Supporting Information

Combining Photo-Organo Redox- and Enzyme Catalysis Facilitates Asymmetric C-H Bond Functionalization

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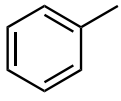
TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AAO	Aryl alcohol oxidase from *Pleurotus eryngii*
ACN	Acetonitrile
ADH	Alcohol dehydrogenase
LB-ADH	Alcohol dehydrogenase from *Lactobacillus brevis*
LK-ADH	Alcohol dehydrogenase from *Lactobacillus kefir*
Ras-ADH	Alcohol dehydrogenase from *Ralstonia* sp.
SY-ADH	Alcohol dehydrogenase from *Sphingobium yanoikuyae*
(*R*)-ATA	Amine transaminase from *Gibbrella zeae*
(*S*)-ATA	Amine transaminase from *Silicobacter pomeroyi*
BAL	Benzaldehyde lyase
CHMO	Cyclohexanone monooxygenase from *Acinetobacter calcoaceticus*
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
GDH	Glucose dehydrogenase from *Pseudomonas* sp.
HAPMO	Hydroxyacetophenone monooxygenase from *Pseudomonas fluorescens*
LDH	L-Lactate dehydrogenase from rabbit muscle
(*R*)-HNL	Hydroxynitrile lyase from *Prunus amygdalus*
(*S*)-HNL	Hydroxynitrile lyase from *Manihot esculenta*
SAS	Sodium anthraquinone-2-sulfate
ERED	Ene-reductase from *Bacillus subtilis* (YqjM)

LIST OF SUBSTRATES/INTERMEDIATES/PRODUCTS

LIST 1: ALKANE SUBSTRATES



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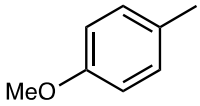

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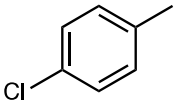

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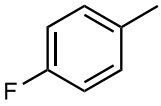

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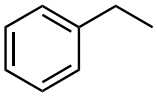

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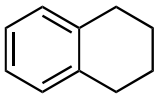

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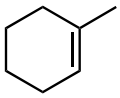

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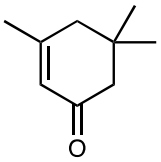

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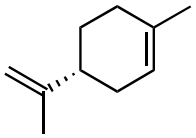

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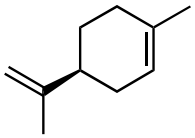

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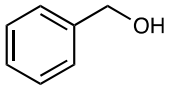
11

LIST 2: ALCOHOL INTERMEDIATES/SUBSTRATES



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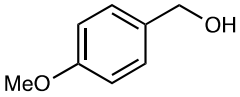

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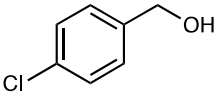

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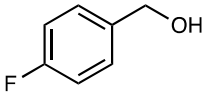

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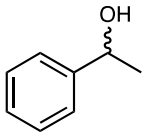

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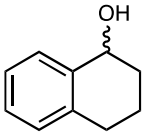

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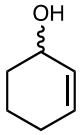

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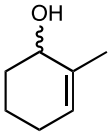

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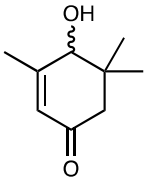

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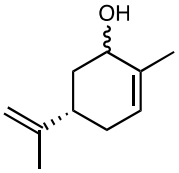

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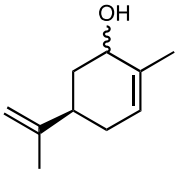

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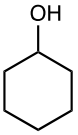

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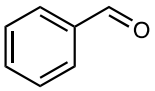
LIST 3: ALDEHYDES/KETONES INTERMEDIATES/SUBSTRATES



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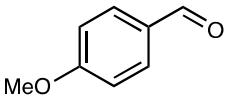

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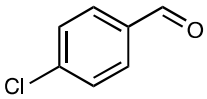

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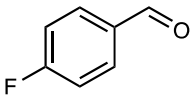

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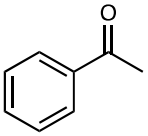

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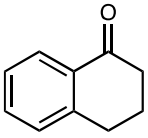

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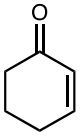

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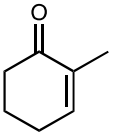

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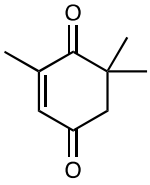

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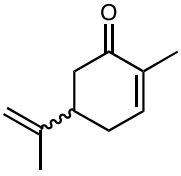

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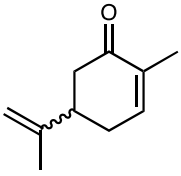

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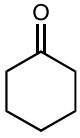

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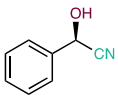
LIST 3: PRODUCTS



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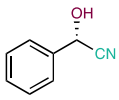

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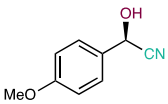

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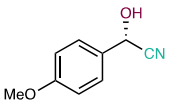

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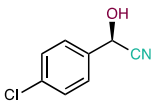

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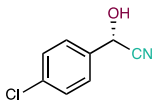

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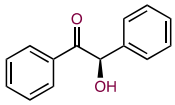

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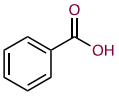

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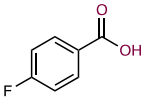

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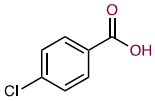

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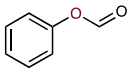

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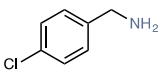

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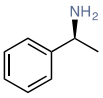

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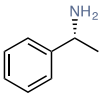

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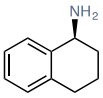

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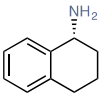

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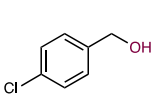
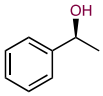
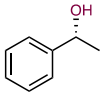


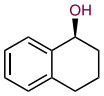
34



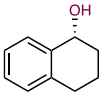
(*R*)-35



(*S*)-35

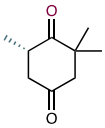


(*R*)-36

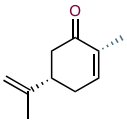


(*S*)-36

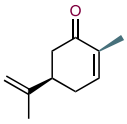
37



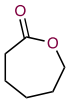
(*S*)-40



(2*R*)-38



(2*S*)-39



41

EXPERIMENTAL PROCEDURES

GENERAL

Unless stated otherwise, all chemicals were purchased from Merck (Sigma-Aldrich, Steinheim, Germany), Fluka (Buchs, Switzerland), New England Biolabs (Ipswich, MA, USA) or Merck (Darmstadt, Germany).

^{1}H and ^{13}C NMR spectra were recorded on a Bruker (300 MHz) instrument, and are internally referenced to residual proton signals in $CDCl_3$. Data for ^{1}H NMR are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, brs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, dt = doublet of triplet, ddd = doublet of doublet of doublet), coupling constant (Hz), and integration. Data for ^{13}C NMR are reported in terms of chemical shift relative to $CDCl_3$ or DMSO-d_6.

BACTERIAL STRAINS AND PLASMIDS

For enzyme production, the following *E. coli* strains were used: *E. coli* BL21 (DE3) [*fhuA2* [*lon*] *ompT gal* (*λ DE3*) [*dcm*] *ΔhsdS*], *E. coli* C43 (DE3) [F – *ompT hsdSB (rB- mB-) gal dcm* (DE3)], E. coli W3110 [F^{-} λ^{-} *rph-1 INV(rrnD, rrnE)*] and *E. coli* Lemo (DE3) [*fhuA2 [lon] ompT gal (λ DE3) [dcm] ΔhsdS/ pLemo*(Cam^{R})].

The genes encoding the ADHs from *Sphingobium yanoikuyae* (SY-ADH, GenBank: 595588160) and *Ralstonia* sp. (Ras-ADH, Genbank: ACB78191) were inserted into pET26b.[1] The genes encoding for the ADH from *Lactobacillus kefir* (LK-ADH, GenBank: AY267012.1) and the ADH from *Lactobacillus brevis* (LB-ADH, GenBank: KIR09363.1), respectively were inserted into pET21a.[2] These ADHs were kindly provided by Prof. W. Kroutil (University of Graz, Austria). The gene for the ene-reductase from *Bacillus subtilis* (YqjM, GenBank: BAA12619.1) was inserted into pET28a. The mature AAO cDNA (GenBank: AF064069) was cloned in pFLAG1.[3] The AAO was provided by Prof. A. T Martinez (CIB, CSIC, Spain).

COMMERCIALLY OBTAINED ENZYMES

Lyophilized Amine transaminases (ECS-ATA01 from *Aspergillus fumigatus*, ECS-ATA08 from *Silicibacter pomeroyi*) and Baeyer-Villiger monooxygenases (CHMO: ECS-Mo01 from *Acinetobacter calcoaceticus*, HAPMO: ECS-Mo05 from *Pseudomonas fluorescens*) were obtained from Enzymicals AG (Greifswald, Germany). One Unit corresponds to the amount of enzyme that releases 1 µmol product per minute at 30˚C under assay conditions specified by the supplier. Lyophilized benzaldehyde lyase (BAL, evo-1.4.106; ≥ 1.6 U/g_{Lyo}) was obtained from Evocatal GmbH (Monheim am Rhein, Germany). One unit (U) is defined as the conversion of 1 µmol furfural per minute at pH 8.0. All other enzymes used were produced in house as specified below. Semi-purified (R)-HNL from *Prunus amygdalus* was obtained from Jülich Fine Chemicals (now Codexis, California, USA). A CLEA of *Manihot esculenta* (*S*)-hydroxynitrile lyase [batch number CLEA MEHNL-S03–150–03512] from CLEA Technologies (Delft, The Netherlands) was used in the synthetic hydrocyanation experiments.[1]

IN-HOUSE PRODUCTION OF ENZYMES

Tab. S1: Cultivation conditions for all in-house produced enzymes. Kan – kanamycin, Amp – ampicillin.

Enzyme	*E. coli* strain	Medium	Antibiotic [concentration]	Inducer [concentration]	Additive [concentration]
SY-ADH	BL21 (DE3)	TB	Kan [50 µg/mL]	IPTG [120 mg/L]	
Ras-ADH	BL21 (DE3)	TB	Kan [50 µg/mL]	IPTG [120 mg/L]	$CaCl_2$ [0.6 mM]
LK-ADH	Lemo (DE3)	TB	Amp [50 µg/mL]	IPTG [1 mM]	
LB-ADH	C43 (DE3)	TB	Amp [50 µg/mL]	Anhydrotetracycline [0.4 µM]	$MgCl_2$ [1 mM]
YqjM	BL21 (DE3)	ZYM-5052	Kan [50 µg/mL]	Autoinduction with lactose	
AAO	W3110	TB	Amp [50 µg/mL]	IPTG [1 mM]	

a) Expression of the ADHs

Expression of the (*S*)-selective SY-ADH and Ras-ADH were carried out by inoculation of 400 mL TB media supplied with the appropriate antibiotic (Tab. S1) and 0.6 mM $CaCl_2$ (in case of Ras-ADH) with an overnight culture to give an OD_{600} of 0.05. *E. coli* BL21 (DE3) cells were used for the expression of these ADHs. Cells were grown at 37°C in baffled shake flasks. ADH expression was then induced at an OD_{600} of 0.6-0.8 with 120 mg/L IPTG (isopropyl β-D-thiogalactopyranoside). For both, cultivation was continued at 20°C for 20 hours.

Expression of the (*R*)-selective LK-ADH and LB-ADH were carried out by inoculation of 400 mL TB media supplied with the appropriate antibiotic (Tab. S1) and 1 mM $MgCl_2$ (in case of LB-ADH) with an overnight culture to give an OD_{600} of 0.05. *E. coli* BL21 (DE3) cells were used for the expression of these ADHs. Cells were grown at 37°C in baffled shake flasks. Expression of LK-ADH was induced at an OD_{600} of 0.6 with 1 mM IPTG, whereas expression of LB-ADH was induced at an OD_{600} of 0.6 with 0.4 mM anhydrotetracyclin. For both, cultivation was continued at 20°C for 20 hours.

Cells were harvested by ultracentrifugation (1344 x *g*, 15 min, 4°C), washed with potassium phosphate buffer (50 mM, pH 7.5) and centrifuged again with the same speed. Subsequently, the cell pellet was resuspended in the same buffer to give a wet cell weight (WCW) of 100 g_{WCW}/L. For cell disruption, the cell suspension was passaged twice through a French pressure cell at 1400 psi. Cell debris was separated from the crude extract by centrifugation at 9000 x *g* for 60 min. The enzymes were then used as crude cell extract in the biocatalysis reactions.

Enzyme activity measurements for all ADHs were performed spectrophotometrically using the Agilent Technologies Cary 60 UV-Vis spectrophotometer (equipped with a single cell Peltier accessory) by monitoring the NADH/NADPH consumption at 340 nm for 120 s in 1 mL

cuvettes. The activity of ADH was determined against acetophenone in potassium phosphate buffer (50 mM, pH 7.5) at 30˚C using a substrate concentration of 1 mM and 0.3 mM NADH/NADPH. A volume of 1-20 µl crude cell extract solution (diluted 1:10 if necessary) was used and the total volume of the reaction mixture was adjusted to 1 mL. The volumetric activities were calculated using an extinction coefficient of ε=6.3 $mM^{-1}cm^{-1}$ (Tab. S2).

Tab. S2: Activity of all produced ADHs against acetophenone as standard substrate.

ADH	Volumetric activity [U/mL]
SY-ADH	0.51
Ras-ADH	2.13
LK-ADH	20.8
LB-ADH	0.46

b) Expression, characterization and purification of YqjM

Expression of YqjM was carried out by inoculating 1 L of autoinduction ZYM-5052 media supplied with the appropriate antibiotic (Tab. S1) with 100 mL of overnight culture. *E. coli* BL21 (DE3) cells were used for the expression of YqjM. Cells were grown overnight at 37°C in baffled shake flasks.

Cells were harvested by centrifugation (10000 *x g*, 15 min, 4˚C), washed with potassium phosphate buffer (20 mM, pH 6.5) and centrifuged again with the same speed. Subsequently, the cell pellet was resuspended in the same buffer. Mechanical cell disruption was effected by using French press. Cell debris were separated from the crude extract by centrifugation at 10000 x *g* for 30 min at 4˚C. Supernatant was loaded to a 14 mL NiNTA chromatography column. The loading was performed using NGC™ Chromatography system (Bio-Rad). After loading, various successive washing steps with 20 mM potassium phosphate buffer pH 6.5 containing 30 mM imidazole were performed in order to elute unspecifically bound proteins. Then, the elution of YqjM was performed by using elution buffer (20 mM potassium phosphate, 250 mM imidazole, pH 6.5) in a gradient 0-100% during 7 column volumes. Fractions containing proteins were tested with the UV assay (reduction of 2-cyclohexen-1-one) and those containing YqjM were collected and incubated with 5 mM FMN. After 30 min of incubation on ice, enzyme suspension was desalted twice using PD-10 Desalting Columns (GE Healthcare), and concentrated using Amicon® Ultra-15 Centrifugal Filter Device (cutoff 30K).

YQJM ACTIVITY MEASUREMENTS

Activity measurements for YqjM was performed spectrophotometrically using the Agilent Technologies Cary 60 UV-Vis spectrophotometer (equipped with a single cell Peltier accessory) by monitoring the NADPH consumption at 340 nm in 1 mL cuvettes. The activity of YqjM was determined against 2-cyclohexen-1-one in potassium phosphate buffer (50 mM, pH 6.5) at 25˚C using a substrate concentration of 1 mM and 0.15 mM NADPH. The volumetric activities

were calculated using an extinction coefficient of ε=6.3 $mM^{-1}cm^{-1}$. Measured specific activity of YqjM was 1.8 ± 0.01 U/mg.

c) Expression, characterization and purification of AAO

The coding sequence of aryl alcohol oxidase (AAO) from *Pleurotus eryngii* was cloned in the expression vector pFLAG1 (International Biotechnologies Inc.) and the resulting plasmid pFLAG1-AAO was used for expression.[3] The AAO was produced in *E. coli* W3110. Cells were grown for 3 h in terrific broth, induced with 1 mM IPTG and grown further for 4 h. Cells were harvested by centrifugation at 10.000 *x g* for 10 minutes at 4°C. Bacteria were resuspended in 100 ml lysis buffer (50 mM Tris-HCl pH 8.0 containing 1 mM EDTA, and 5 mM DTT), supplemented with lysozyme (2mg/ml) and DNAseI. After 1 hour of incubation, cells were sonicated and debris was removed by centrifugation at 10.000 *x g* for 1 hour. The apoenzyme accumulated in inclusion bodies, as observed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and was solubilized with 8 M urea. *In vitro* refolding was performed with 0.2 mg/mL protein by using 0.6 M urea, 20 µM FAD, 2.5 mM oxidized glutathione, 1 mM dithiothreitol at pH 9 in 34% glycerol. The AAO was purified by Resource-Q chromatography using a 0-1 M NaCl gradient (2 ml/min, 20 min) in 10 mM sodium phosphate (pH 6.0). AAO concentration was determined using the molar absorption coefficient (ε_{463} = 11050 M^{-1} cm^{-1}).

AAO ACTIVITY MEASUREMENTS

The AAO activity was measured spectrophotometrically using the Agilent Technologies Cary 60 UV-Vis spectrophotometer (equipped with a single cell Peltier accessory) by monitoring the oxidation of veratryl alcohol (VA, 3,4-dimethoxybenzylalcohol) to veratraldehyde at 310 nm. The reaction mixture contained 8 mM of VA in 100 mM sodium phosphate buffer, pH 6.0. One unit of AAO activity is defined as the amount of enzyme that converts 1 µM of alcohol to aldehyde per minute at 25 °C.

ANALYTICS

GC analysis - General

All measurements have been performed at least as duplicates. Numbers shown are always based on calibration curves with authentic standards of the reagents (using internal standards). Error bars shown represent the standard deviation.

In general, samples from the concurrent monophasic reactions were taken at defined time intervals and extracted with the same volume of ethyl acetate (containing 5 mM dodecane or 1-octanol as internal standard). For the concurrent biphasic and stepwise biphasic cascade reactions, samples have been taken at defined time intervals from the aqueous as well as the organic phase. Samples of the organic phase were extracted with a 40-fold higher volume (sample dilution for the two-phasic reactions) of ethyl acetate (containing 5 mM dodecane, 1-octanol or acetophenone as internal standard). If no derivatization of the obtained products was necessary, the organic phase was directly transferred into a clean tube and dried over

anhydrous $MgSO_4$. The solution was again mixed (60 sec) and centrifuged (10 min). Afterwards, samples were transferred into GC vials and 1 µl sample was injected into the GC instrument. Analysis was performed using a Shimadzu GC-14A or GC-2010 equipped with the columns specified in Tab. S3 and flame-ionization detection (FID). Concentrations have been determined based on calibration curves using authentic standards.

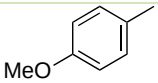
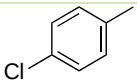
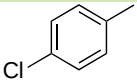
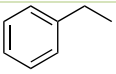
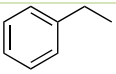
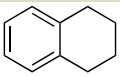
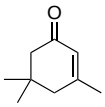
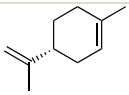
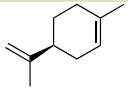
HPLC analysis

All measurements have been performed at least as duplicates. Numbers shown are always based on calibration curves with authentic standards of the reagents. Error bars shown represent the standard deviation.

At regular time intervals, aliquots from the organic phase were diluted 1:25 in heptane/isopropanol (80:20 for the acids and 50:50 for benzoin) and aliquots from the aqueous phase were extracted 1:1 in heptane/isopropanol (80:20 for the acids and 50:50 for Benzoin) and analysed by HPLC. HPLC measurements were performed on a Shimadzu LC-20 system with a Shimadzu mSPD-20A Photo Diode Array detector using Chiralpak AD-H column (5 µM, 4.6×250 mm) and conditions specified in Tab. S4.

Tab. S3. Details of GC analysis. [a]

Substrate	Column	T_R [min]	Temperature profile

 **1**	Column B	**30**: 8.35	80°C hold 4 min; 20°C/min to 150°C hold 2.50 min; 25°C/min to 220°C hold 4 min
 **1**	Column A	(***R***)-**23**: 7.84 [b] (***S***)-**23**: 8.21[b]	125 °C hold 3 min, 20 °C /min to 185 °C hold 2.5 min, 30 °C /min to 225 °C hold 1 min.
 **2**	Column A	(***R***)-**24**: 12.06[b] (***S***)-**24**: 12.65[b]	120 °C hold 4 min, 20 °C /min to 180 °C hold 5.8 min, 25 °C /min to 225°C hold 1.5 min.
 **3**	Column A	(***R***)-**25**: 11.65[b] (***S***)-**25**: 11.99[b]	120 °C hold 4 min, 10 °C /min to 130 °C hold 0 min, 15 °C /min to 180°C hold 3.8 min, 25 °C /min to 225°C hold 1.5 min.
 **3**	Column C	**31**: 13.20 **34**: 4.7	70°C hold 2 min; 15°C/min to 95°C hold 2.8 min, 20°C/min to 110°C hold 1.0 min, 20°C/min to 150°C hold 2.50 min, 25°C/min to 240°C hold 2.50 min
 **5**	Column A	(***R***)-**32**: 11.35 (***S***)-**32**: 11.55	125°C hold 3 min; 10°C/min to 140°C hold 3.10 min; 15°C/min to 180°C hold 2.50 min; 25°C/min to 225°C hold 1 min
 **5**	Column A	(***R***)-**35**: 14.96 (***S***)-**35**: 15.95	100 °C hold 4 min, 10 °C /min to 120 °C hold 10 min, 25 °C /min to 215 °C hold 1.3 min
 **6**	Column A	(***R***)-**33**: 12.05 (***S***)-**33**: 12.55 (***R***)-**36**: 11.11 (***S***)-**36**: 10.98	130°C hold 2 min; 10°C/min to 150°C hold 4 min, 10°C/min to 165°C hold 3.50 min, 25°C/min to 225°C hold 1 min
 **8**	Column B	**37**: 7.09	100 °C hold 3 min, 5 °C /min to 135 °C hold 0 min, 15 °C /min to 160 °C hold 1.5 min, 15 °C /min to 180 °C hold 1 min, 25 °C /min to 210 °C hold 1.5 min.
 **9**	Column B	(***S***)-**40**: 20.75	100 °C hold 3.5 min, 5 °C /min to 125 °C hold 12.2min, 25 °C /min to 210 °C hold 1.5 min.
 (***R***)-**10**	Column B	(**2*R*)-38**: 11.12	100 °C hold 3.5 min, 10 °C /min to 160 °C hold 2.6 min, 25 °C /min to 210 °C hold 1.5 min.
 (***S***)-**10**	Column B	(**2*S*)-39**: 10.70	100 °C hold 3.5 min, 10 °C /min to 160 °C hold 2.6 min, 25 °C /min to 210 °C hold 1.5 min.
 **11**	Column D Column B	**41**: 9.78	90°C hold 3 min; 20°C/min to 100°C hold 4.50 min; 30°C/min to 230°C hold 1 min 125°C hold 3 min; 15°C/min to 180°C hold 3.30 min; 25°C/min to 220°C hold 1 min

[a] Quantification of non-chiral compounds (alkanes and aldehydes/ketones) was reported in previous procedures. Column A: ChiralsilDex CB column (25 m × 0.32 mm × 0.25 µm), FID, helium is the carrier gas. Column B: LIPODEX E column (50 m × 0.53 mm × 2 µm), FID, helium is the carrier gas. Column C: CP Sil 5CB column (50 m × 0.53 mm × 1 µm), FID, helium is the carrier gas. Column D: CP Wax 52 CB column (50 m × 0.53 mm × 2 µm), FID, helium is the carrier gas. [b] In order to measure the *ee*, 3 mg of *N,N*-Dimethylpyridin-4-amine (DMAP) and 10 uL of acetic anhydride were added to the ethyl acetate containing dodecane (after the exaction of the samples). The mixture was kept at 30 °C for 45 minutes, then 100 uL of MilliQ water was added to stop the acetylation. The organic phase was dried over $MgSO_2$ and measured.

Tab. S4. Details of HPLC analysis.

Substrate	Product(s)	Method	Column	Flow

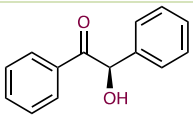
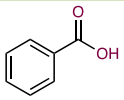
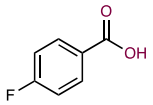
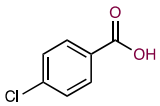
 **1**	 **(*R*)-26**	7 % B for 25 min; Solvent A: heptane; Solvent B: isopropanol; UV detection at 215 nm	Chiralpak AD-H column (5 µM, 4.6×250 mm)	1.0 mL/min
 **1**	 **27**  **28**  **29**	5 % B for 16 min; Solvent A: heptane; Solvent B: isopropanol; UV detection at 215 nm	Chiralpak AD-H column (5 µM, 4.6×250 mm)	1.0 mL/min

PHOTO-CHEMO-ENZYMATIC CASCADE REACTIONS IN GENERAL

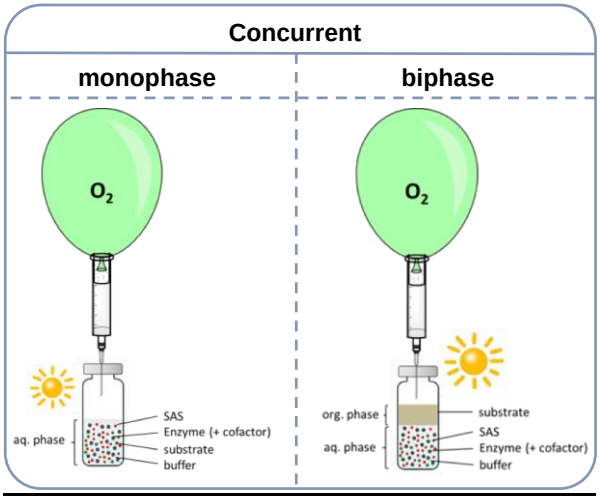


Fig. S1: Schemes for different types of photochemo-enzymatic cascade reactions. Concurrent cascade mode either in a monophasic (left) manner or as biphasic (right) system. Note that the needle supplying the oxygen was placed in the air phase above the liquid.

CONCURRENT

a) Monophase

For the concurrent monophasic reactions (Fig. 1 left), the photochemo-enzymatic cascades were performed simultaneously in the buffer necessary for the respective enzyme. The reaction solution contained the SAS catalyst (1 mM) the enzyme, the enzyme's cofactor if necessary, the substrate and a co-solvent. The reaction solution was supplied with pure oxygen as well as light exposure during the whole reaction.

b) Biphase

For the concurrent biphasic reactions (Fig. 1 right), the photochemo-enzymatic cascades were also performed simultaneously. However, the reaction was taking place at the interface of organic (containing the respective substrate) and aqueous phase, which contained the respective enzyme. The aqueous phase consisted of the SAS catalyst (1 mM), the enzyme and the enzyme's cofactor if necessary. The reaction solution was supplied with pure oxygen as well as light exposure during the whole reaction.

STEPWISE

For the stepwise biphasic reactions, the photochemo-enzymatic cascades were performed in a sequential running mode. First, the light-driven oxidation of the substrates using the photochemo-catalyst SAS in a two-phase system were performed. After 24 h, the SAS catalyst present in the aqueous phase was exchanged by a new aqueous phase containing the selected enzyme. In practice, we transferred the organic phase containing the product from the first step into a new reaction vessel, which contained the freshly prepared aqueous phase with the respective enzyme, cofactor and buffer was added. After addition of the organic phase, the enzymatic reaction step was performed.

GENERAL PROCEDURE FOR THE PHOTOOXIDATION OF ALKANES AND ALCOHOLS

The photooxidation reactions were performed by using a homemade light-setup at 30°C under oxygen atmosphere (supplied by oxygen balloons). All reactions were exposed to visible light bulb (Philips 7748XHP 200W, white light bulb) and continuously stirred at 200 rpm. The distance between the light bulb and the reaction vial was 3.6 cm. The homemade experimental setup is shown in Fig. S2. In order to perform one-pot cascade reactions, components necessary for enzymatic reactions were added to the same vial. In case of the stepwise biphasic reactions, first 700 µL of MilliQ water (containing 1.0 mM of SAS) and 300 µL of organic substrates were added to a 4 mL glass vial. While this type of cascade reactions, the aqueous phase was removed and a freshly prepared aqueous phase containing the components for the enzymatic reactions was added to the same vial. At intervals, samples were taken from the aqueous and organic phase, extracted/diluted with ethyl acetate (containing 5 mM of dodecane, acetophenone or 1-octanol as internal standards), dried over anhydrous $MgSO_4$ and analysed by GC (Shimadzu) employing the columns and temperature profiles listed in Tab. S3 (GC) and S4 (HPLC).

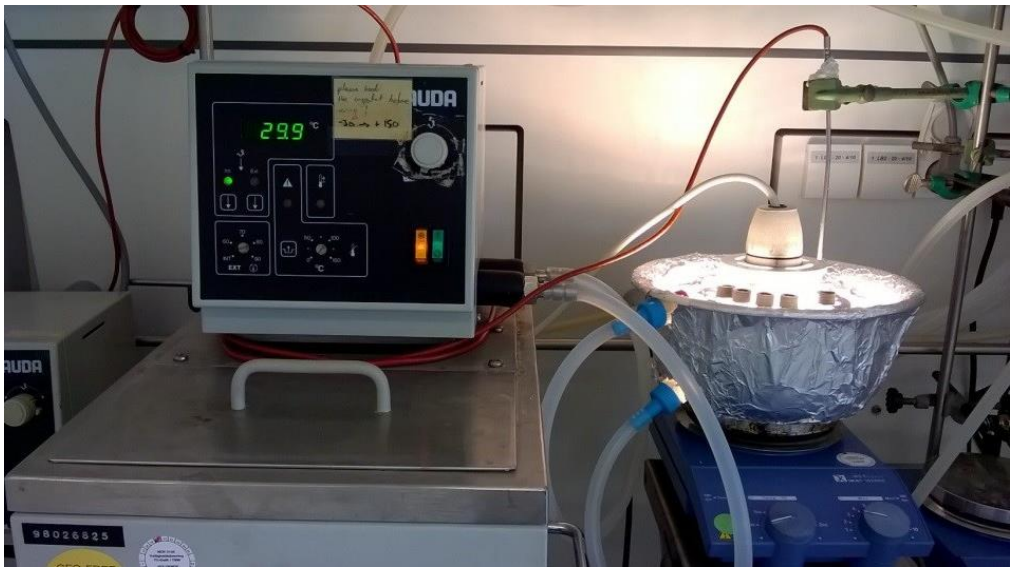


Fig. S2: Image of the homemade photocatalytic setup.

NADH AND NADPH OXIDATION BY SAS

The oxidation of NADH by SAS was investigated by measuring the decrease of absorption at 340 nm for 22 hours. A solution of 30 mM NADH in Sodium phosphate buffer (pH 7.5, 50 mM) with and without SAS (1 mM) was incubated at 30˚C under light and oxygen supply. The oxidation of NADPH by SAS was investigated by measuring the decrease of absorption at 340 nm for 22 hours. A solution of 25 mM NADPH in 50 mM potassium phosphate buffer at pH 9.5 (typical pH for enzymatic steps used) with and without SAS was incubated at 30˚C under light and oxygen supply.

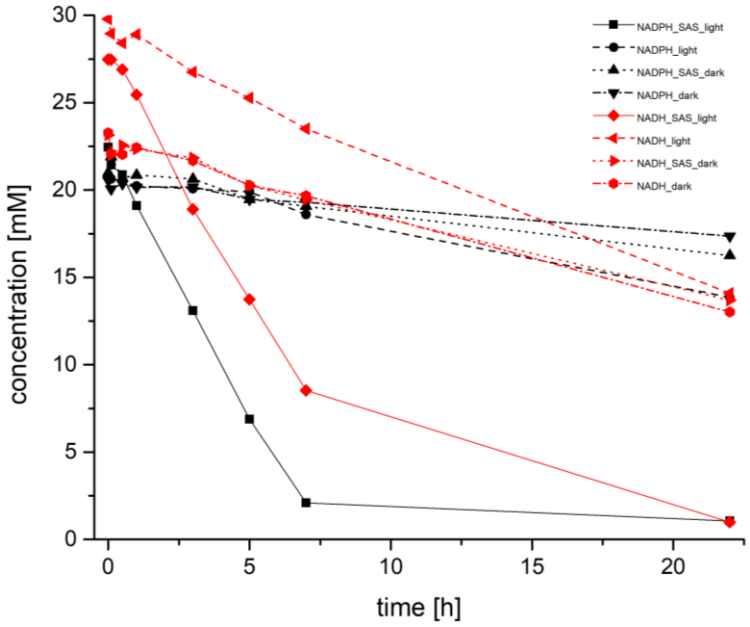


Fig. S3: Decrease of NADH with SAS (red lines) and NADPH (green lines) with and without SAS and light, respectively over 22 hours.

DETAILS ABOUT PHOTO-CHEMO-ENZYMATIC CASCADE REACTIONS SORTED BY ENZYMATIC REACTIONS

1. BAEYER-VILLIGER MONOOXYGENASES (BVMOS)

The monophasic and biphasic concurrent as well as the biphasic stepwise photo-chemo-enzymatic cascades have been performed as described above. The details about the reaction composition and reaction conditions applied for each cascade mode are displayed in Tab. S5.

Tab. S5. Details about photo-chemo-enzymatic reactions catalysed by BVMOs.

Cascade mode	Reaction mode	Substrate	Enzyme	Reaction composition	Reaction conditions for SAS oxidation	Reaction conditions for enzymatic step
concurrent	monophase		**HAPMO**	1 mM SAS, 0.1 mM FAD, 40 mM NADPH, 1 mg HAPMO lyophilisate, KP_i (50 mM, pH 8.0), 10% (v/v) ACN, 20 mM substrate, V_{final} = 1 mL	O_2 atmosphere, T=30°C, λ>400 nm, 24 h	
	biphase			1 mM SAS, 0.1 mM FAD, 25 mM NADPH, 1 mg HAPMO lyophilisate, KP_i (50 mM, pH 8.0), alkane/water [$c(SAS)_{aq}$ = 1 mM]=3:7 (300 µL org. phase from the alkane oxidation), V_{final} = 1 mL		
	monophase		**CHMO**	1 mM SAS, 0.1 mM FAD, 40 mM NADPH, 1 mg CHMO lyophilisate, KP_i (50 mM, pH 8.0), 10% (v/v) ACN, 20 mM substrate, V_{final} = 1 mL		
	biphase			0.1 mM FAD, 25 mM NADPH, 1 mg CHMO lyophilisate, KP_i (50 mM, pH 8.0), alkane/water [$c(SAS)_{aq}$ = 1 mM]=3:7 (300 µL org. phase from the alkane oxidation), V_{final} = 1 mL		
stepwise	biphase		**HAPMO**	0.1 mM FAD, 25 mM NADPH, 1 mg HAPMO lyophilisate, KP_i (50 mM, pH 8.0), alkane/water [$c(SAS)_{aq}$ = 1 mM]=3:7 (300 µL org. phase from the alkane oxidation), V_{final} = 1 mL	O_2 atmosphere, T=30°C, λ>400 nm, 24 h	O_2 atmosphere, 25°C, 20-22 h, 800 rpm
			**CHMO**	0.1 mM FAD, 25 mM NADPH, 1 mg CHMO lyophilisate, KP_i (50 mM, pH 8.0), alkane/water [$c(SAS)_{aq}$ = 1 mM]=3:7 (300 µL org. phase from the alkane oxidation), V_{final} = 1 mL		

Exemplary time course analysis of reactions employing BVMOs

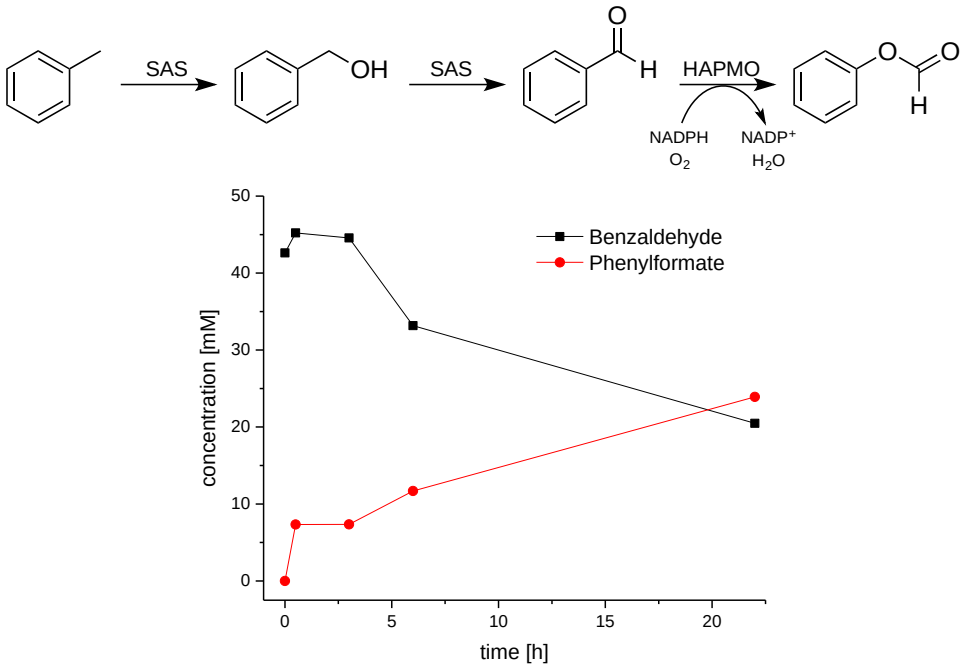


Fig. S4: Time course of the second step of the stepwise biphasic oxidation of toluene to phenyl formate. The HAPMO-catalysed conversion of benzaldehyde to phenyl formate was monitored for 22 hours. Reaction conditions are depicted in Tab. S5.

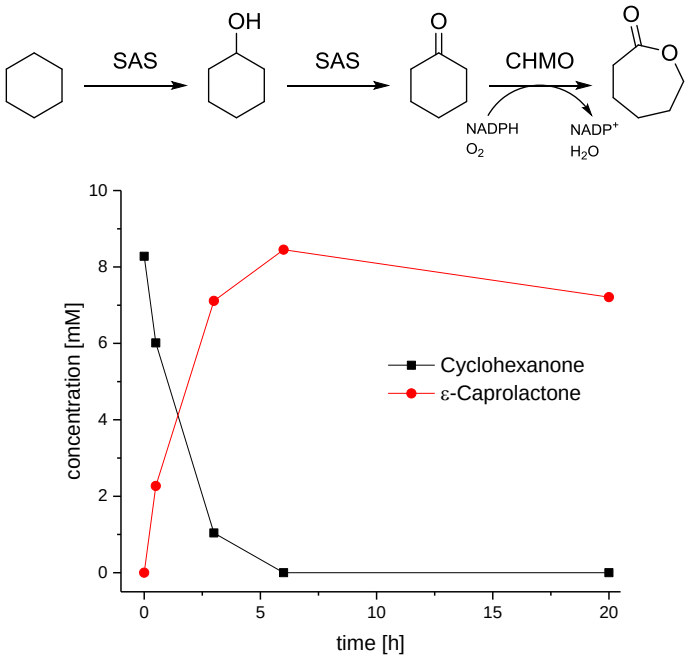
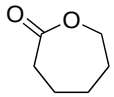
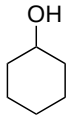
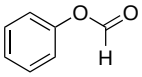
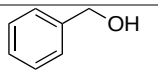


Fig. S5: Time course of the second step of the stepwise biphasic oxidation of cyclohexane to ε-caprolactone. Reaction conditions are depicted in Tab. S5.

Summary about BVMO-catalysed reactions

Tab. S6. Summary about all the different reactions starting from different substrates using BVMOs.

Enzyme	Starting from	Product	Control Reaction[a]	Concurrent monophase	Concurrent biphase[b]	Stepwise biphase[c]
				Product Concentration [mM]		
CHMO			20.3 (> 99% conversion)	n.d.	n.d.	8.5 (7.2)
				2.0	5.0	19
HAPMO			17.75 (> 89% conversion)	n.d.	23.5	23.9
				9.5	31.4	31.2

[a] Starting from 20 mM substrate concentration; [b] NADPH concentration was 40 mM; [c] NADPH concentration was 25 mM. n.d. not detected.

2. HYDROXYNITRILE LYASES (HNLS)

The monophasic and biphasic concurrent as well as the biphasic stepwise photo-chemo-enzymatic reactions have been in general performed as described above. The details about the reaction composition and reaction conditions applied for each reaction modes are displayed in Tab. S7.

Tab. S7. Details about photo-chemo-enzymatic reactions catalysed by HNLs.

Cascade mode	Reaction mode	Substrate	Enzyme	Reaction composition	Reaction conditions for SAS oxidation	Reaction conditions for enzymatic step
concurrent	monophase		**(*R*)-HNL**	125 µL HCN (stock 2M) was added to 785 µL of 1 mM SAS in 50 mM citrate buffer (pH 4.0), 100 µL of ACN, 1 mg/mL of *Pa*HNL and 2.7 µL toluene (25 mM end concentration), V_{final} = 1 mL	O_2 atmosphere, T=30°C, λ>400 nm, 24 h	
	biphase			50 µL HCN (stock 2M) was added to 700 µL of 1 mM SAS in 50 mM citrate buffer (pH 4.0), 1 mg/mL of *Pa*HNL and 300 µL toluene, V_{final} = 1 mL		
	monophase		**(*S*)-HNL**	125 µL HCN (stock 2M) was added to 785 µL of 1 mM SAS in 50 mM citrate buffer (pH 4.0), 100 µL of ACN, 1 mg/mL of *Me*HNL and 2.7 µL toluene (25 mM), V_{final} = 1 mL		
	biphase			50 µL HCN (stock 2M) was added to 700 µL of 1 mM SAS in 50 mM citrate buffer (pH 4.0), 1 mg/mL of *Me*HNL and 300 µL toluene, V_{final} = 1 mL		
Stepwise, One-pot	biphase,		**(*R*)-HNL**	700 µL of 1 mM SAS in 50 mM citrate buffer (pH 4.0) was added to 300 µL toluene. The reaction was supplied with	O_2 atmosphere, T=30°C, λ>400 nm, 24 h	30°C, 24 h, 200 rpm
			(*S*)-HNL			

	Direct enzyme addition			pure oxygen and light exposure and stirred at 200 rpm for 24 hours. The vial was then protected from light with aluminium foil and 60 µL HCN (stock 2 M) and 1 mg/mL of *Pa*HNL or *Me*HNL were added.		
Stepwise, With phase transfer	biphase, Enzyme addition in new aqueous phase		**(*R*)-HNL** **(*S*)-HNL**	700 µL of 1 mM SAS in 50 mM citrate buffer (pH 4.0) was added to 300 µL toluene. The reaction was supplied with pure oxygen and light exposure and stirred at 200 rpm for 24 hours. The reaction mixture was then centrifuged and the organic phase was transferred to a new vial. To this solution, 90 µL HCN (stock 2 M, 10 equivalents) and 1 mg/mL of *Pa*HNL or 1 mg *Me*HNL CLEA were added.	O_2 atmosphere, T=30°C, λ>400 nm, 24 h	30°C, 24 h, 200 rpm

Exemplary time course analysis of reactions employing HNLs

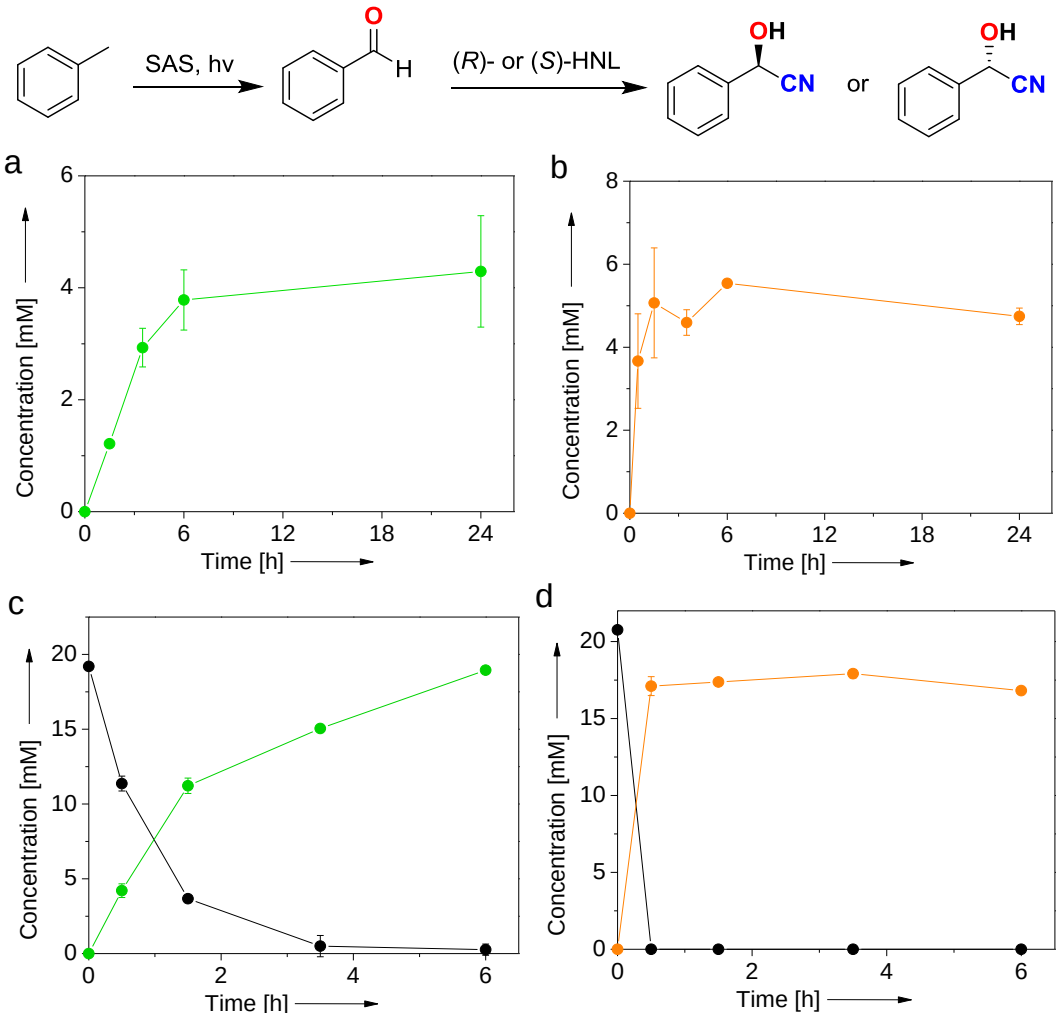


Fig. S6. Time course of the second step of the photo-chemo-enzymatic transformation reaction of toluene to (*R*)- or (*S*)-mandelonitrile. A and B) (*R*)-HNL or (*S*)-HNL was directly added to the aqueous phase of the SAS-reaction after 24 h. C) and D) (*R*)-HNL or (*S*)-HNL were provided as new aqueous phase in a new reaction vessel. Formed benzaldehyde (Black dot) present in the organic phase from the first step was transferred to this vessel.

3. BENZALDEHYDE LYASE (BAL)

The monophasic and biphasic concurrent as well as the biphasic stepwise photo-chemo-enzymatic cascades have been in general performed as described above. The details about the reaction composition and reaction conditions applied for each cascade mode are displayed in Tab. S8.

Tab. S8. Details about photo-chemo-enzymatic reactions catalysed by BAL.

Cascade mode	Reaction mode	Substrate	Enzyme	Reaction composition	Reaction conditions for SAS oxidation	Reaction conditions for enzymatic step
concurrent	monophase		**BAL**	1 mM SAS in KPi (50 mM, pH 9.5), 0.5 mM thiaminpyrophosphate (TPP), 0.5 mM $MgCl_2$, 7 mg BAL lyophilisate, 10 mM toluene, V_{final} = 1 mL	O_2 atmosphere, T=30°C, λ>400 nm, 24 h	
stepwise	biphase		**BAL**	First step: 1 mM SAS in KPi (50 mM, pH 9.5) and 300 µL toluene. Second step: 0.5 mM TPP, 0.5 mM $MgCl_2$, 7 mg BAL lyophilizate, KPi (50 mM, pH 9.5), 300 µL org. phase from the SAS Oxidation, V_{final} = 1 mL.	O_2 atmosphere, T=30°C, λ>400 nm, 24 h	30°C, 22 h, 200 rpm

Due to the precipitation of (*R*)-benzoin during the reactions (Fig. S7), the concentration in the aqueous phase determined by HPLC analysis was always low. Thus, after reaction was completed, the whole mixture was extracted three times with ethyl acetate. After evaporation of the solvent, the purity of the obtained product has been determined by HPLC and ^{1}H NMR and concentrations in the reaction have been recalculated using the obtained mass of product.

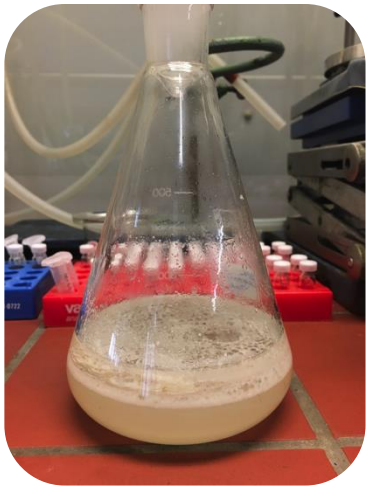
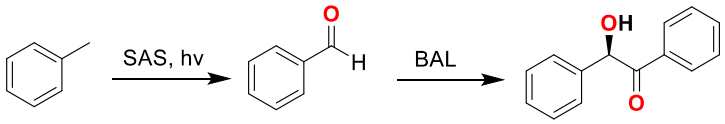
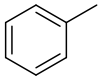
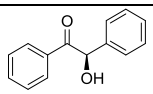
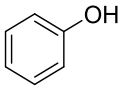


Fig. S7: Close-up of the reaction solution after 22 hours of reaction. The product (*R*)-benzoin is visible as white precipitate at the interface of organic and aqueous phase.

Tab. S9. Summary of reactions starting from different substrates using BAL.



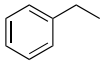
Enzyme	Starting from	Product	Control Reaction[a]	concurrent 1-phase[b]	stepwise[b]
			Product Concentration [mM]		
BAL			18.5 (85 % conversion)	1.5	102
				18.8	89

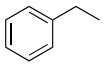
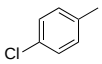
[a] Starting from 22 mM benzaldehyde concentration after 6 hours, [a] Concentrations after work-up are given.

4. AMINE TRANSAMINASES (ATAS)

The monophasic and biphasic concurrent as well as the biphasic stepwise photo-chemo-enzymatic cascades have been in general performed as described above. The details about the reaction composition and reaction conditions applied for each cascade mode are displayed in Tab. S10.

Tab. S10. Details about photo-chemo-enzymatic reactions catalysed by ATAs.

Cascade mode	Reaction mode	Substrate(s)	Enzyme	Reaction composition	Reaction conditions for SAS oxidation	Reaction conditions for enzymatic step
concurrent	monophase	  Cl 	**(*R*)-ATA**	SAS (1 mM), isopropylamine (500 mM), PLP (0.1 mM), (*R*)-ATA (2 mg/mL), NaPi (50 mM, pH 7.5), 10% (v/v) DMF, 25 mM substrate, V_{final} = 1 mL	O_2 atmosphere, T=30°C, λ>400 nm, 16 h	
	biphase			700 µL of SAS (1 mM), isopropylamine (500 mM), PLP (0.1 mM), (*R*)-ATA (2 mg/mL), 10% (v/v) ACN or DMSO, dissolved in NaPi (50 mM, pH 7.5), 300 µL substrate, V_{final} = 1 mL		
	monophase		**(*S*)-ATA**	SAS (1 mM), glucose dehydrogenase (20 U), L-lactate dehydrogenase (95 U), L-alanine (500 mM), pyruvate (50 mM), glucose (150 mM), NADH (5 mM), PLP (0.1 mM), (*S*)-ATA (2 mg/mL), CHES buffer (100 mM, pH 9.0)), 10% (v/v) DMF, 25 mM substrate, V_{final} = 1 mL		
	biphase			700 µL of SAS (1 mM), glucose dehydrogenase (20 U), L-lactate dehydrogenase (95 U), L-alanine (500 mM), pyruvate (50 mM), glucose (150 mM), NADH (5 mM), PLP (0.1 mM), (*S*)-ATA (2 mg/mL), 10% (v/v) DMF		

				dissolved in CHES buffer (100 mM, pH 9.0)), 300 µL substrate, V_{final} = 1 mL		
stepwise	biphase		**(*R*)-ATA**	700 µL of 1 mM SAS in 50 mM NaPi (pH 7.5) was added to 300 µL substrate. The reaction was supplied with pure oxygen and light exposure and stirred at 200 rpm for 24 hours. The reaction mixture was then centrifuged and the organic phase was transferred to a new vial. To this solution, isopropylamine (500 mM), PLP (0.1 mM) and (*R*)-ATA (2 mg/mL) were added.	O_2 atmosphere, T=30°C, λ>400 nm, 24 h	30°C, 24-28 h, 200 rpm
			**(*S*)-ATA**	700 µL of 1 mM SAS in 100 mM CHES (pH 9.0) was added to 300 µL substrate. The reaction was supplied with pure oxygen and light exposure and stirred at 200 rpm for 24 hours. The reaction mixture was then centrifuged and the organic phase was transferred to a new vial. To this solution, glucose dehydrogenase (20 U), L-lactate dehydrogenase (95 U), L-alanine (500 mM), pyruvate (50 mM), glucose (150 mM), NADH (5 mM), PLP (0.1 mM), (*S*)-ATA (2 mg/mL) were added.		
						

Exemplary time course analysis of reactions employing ATAs

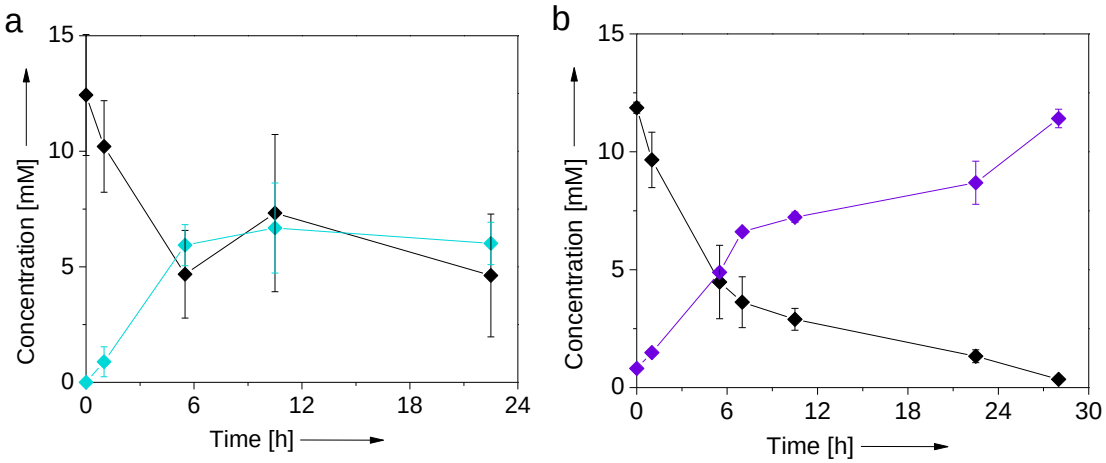


Fig. S8: Time course of the second step of the photo-chemo-enzymatic transformation reaction of ethylbenzene to phenylethanamine in a stepwise reaction mode. After photooxidation of ethyl benzene to acetophenone (◆), a) conversion to (*R*)- phenylethanamine (◆) or b) conversion to (*S*)-phenylethanamine (◆) was catalysed by (*R*)- or (*S*)-selective ATA, respectively.

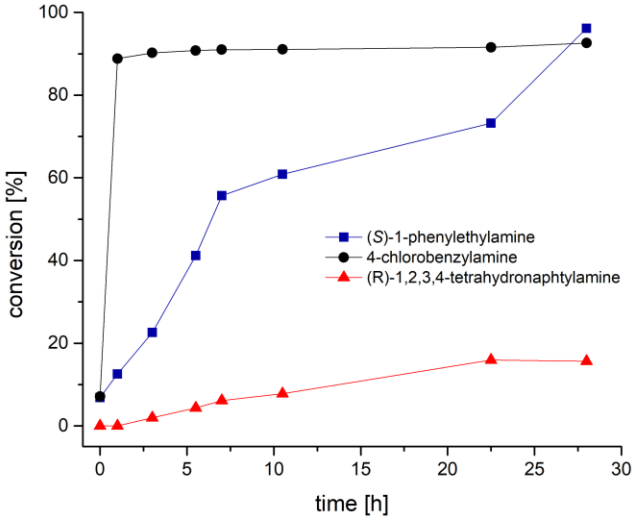


Fig. S9: Conversion over time for the two-step two-pot reaction employing the (*S*)-and (*R*)-selective ATAs over 28 hours. The conversion to (*S*)-(-)-phenylethylamine (●), (*R*)-(-)-1,2,3,4-tetrahydro-1-naphthylamine (▲) as well as the conversion to 4-chlorobenzylamine (■) was followed by taking samples (5 µL) from the organic phase. For (*S*)-phenylethanamine, the calculated concentrations of the amine in the aqueous phase was summed up with the concentrations determined in the organic phase.

Tab. S11. Summary about reactions starting from different substrates using (*R*)-and (*S*)-selective ATAs.

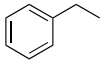
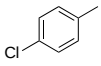
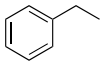
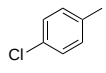
Enzyme	Starting from	Product	Control Reaction[a,c]	Concurrent 1-phase[b]	Concurrent 2-phases	Stepwise
			Product Concentration [mM]			
(*S*)-ATA			9.7 (97.3% conversion)	3.8	0.4	11.5 (96.2% conversion)
				0.44	0.86	0.67
(*R*)-ATA			4.4 (44.7% conversion)	5.53	0.12	6.3 mM (33.3% conversion)
				0.12	0.91	0.724

[a] Starting from 10 mM substrate after 24 hours; [b] 5 % (v/v) DMF added; [c] 10 % (v/v) DMSO added.

5. ALCOHOL DEHYDROGENASES (ADHS)

The monophasic and biphasic concurrent as well as the biphasic stepwise photo-chemo-enzymatic cascades have been in general performed as described above. The details about the reaction composition and reaction conditions applied for each cascade mode are displayed in Tab. S12.

Tab. S12. Details about photo-chemo-enzymatic reactions catalysed by ADHs.

Cascade mode	Reaction mode	Substrate(s)	Enzyme	Reaction composition	Reaction conditions for SAS oxidation	Reaction conditions for enzymatic step
concurrent	monophase one-pot	  	**(*R*)-ADH**	SAS (1 mM), 30 mM NAD(P)H, 2 mg/mL ADH-T or Ras-ADH, 10% (v/v) ACN, 20 mM substrate in NaPi (50 mM, pH 7.5), V_{final} = 1 mL	O_2 atmosphere, T=30°C, λ>400 nm, 18-24 h	
	biphase one-pot			700 µL of SAS (1 mM), 30 mM NAD(P)H, 2 mg/mL ADH-T or Ras-ADH dissolved in NaPi (50 mM, pH 7.5), 300 µL substrate, V_{final} = 1 mL		
	monophase one-pot		**(*S*)-ADH**	SAS (1 mM), 30 mM NAD(P)H, 2 mg/mL LK-ADH or LB-ADH, 10% (v/v) ACN, 20 mM substrate in NaPi (50 mM, pH 7.5), V_{final} = 1 mL		
	biphase one-pot			700 µL of SAS (1 mM), 30 mM NAD(P)H, 2 mg/mL LK-ADH or LB-ADH dissolved in NaPi (50 mM, pH 7.5), 300 µL substrate, V_{final} = 1 mL		
stepwise	biphase two-pot	  	**(*R*)-ADH**	700 µL of 1 mM SAS in 50 mM NaPi (pH 7.5) was added to 300 µL substrate. The reaction was supplied with pure oxygen and light exposure and stirred at 200 rpm for 24 hours. The reaction mixture was then centrifuged and the organic phase was transferred to a new vial. To this solution, 20 mM NAD(P)H, 2 mg/mL ADH-T or Ras-ADH in NaPi (50 mM, pH 7.5) were added.	O_2 atmosphere, T=30°C, λ>400 nm, 24 h	30°C, 18-24 h, 200 rpm
			(*S*)-ADH	700 µL of 1 mM SAS in 50 mM NaPi (pH 7.5) was added to 300 µL substrate. The reaction was supplied with pure oxygen and light exposure and stirred at 200 rpm for 24 hours. The reaction mixture was then centrifuged and the organic phase was transferred to a new vial. To this solution, 20 mM NAD(P)H, 2 mg/mL LK-ADH or LB-ADH in NaPi (50 mM, pH 7.5) were added.		

Exemplary time course analysis of reactions employing ADHs

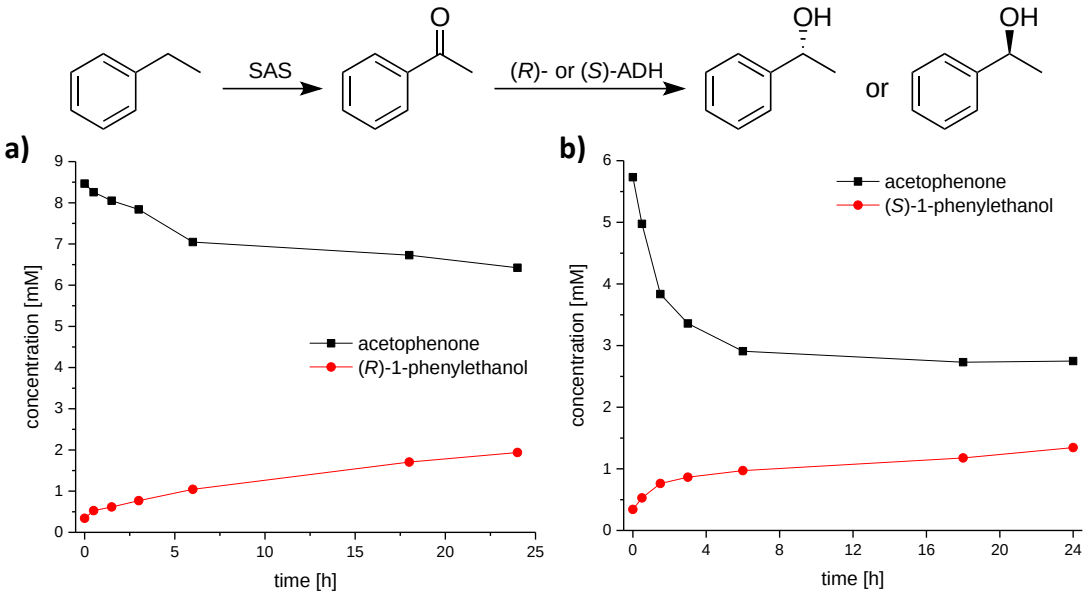


Fig. S10: Time course of the second step of the photo-chemo-enzymatic transformation reaction of ethylbenzene to 1-phenylethanol in a stepwise reaction mode using a) an (*R*)-selective or b) an (*S*)-selective alcohol dehydrogenases. Conversion of acetophenone (■) to a) (*R*)-phenylethanamine or b) (*S*)-phenylethanamine (•) catalysed (*R*)- or (*S*)-selective ADH.

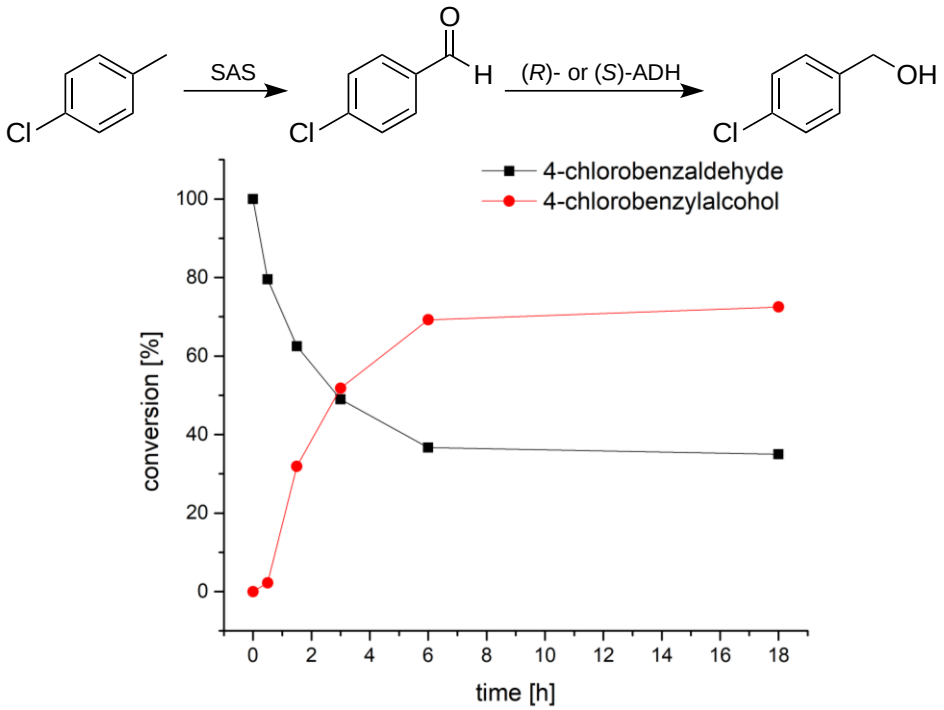


Fig. S11: Time course of the second step of the photo-chemo-enzymatic transformation reaction of p-chlorobenzaldehyde (■) to p-chlorobenzylalcohol (•) in a stepwise reaction mode using LK-ADH for 18 hours.

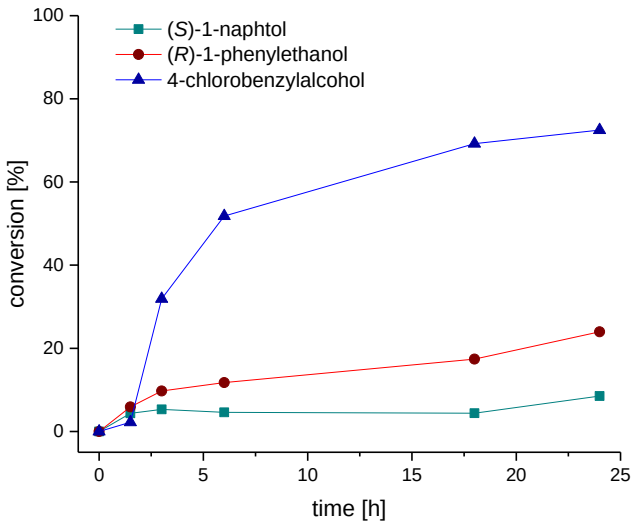
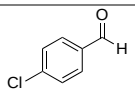
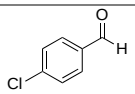


Fig. S12: Comparison of conversions achieved over time for the stepwise reaction employing LK-ADH 18 hours.

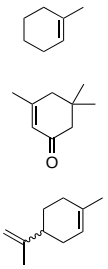
Tab. S13: Summary about the concurrent biphasic reaction to the desired alcohols.

Enzyme	*in situ* generated substrate	Substrate conc. after 18 h [mM]	(*S*)-OH [mM]	(*R*)-OH [mM]	*ee* [%]
LK-ADH		4.5	7.2	8.8	9.5
		122.4	2.8	-	-
		337.2	10.9	-	>99
Ras-ADH		4.5	1.3	1.0	12.6
		250.4	2.9	-	-
		102.8	2.6	0.32	78.4

6. ENE-REDUCTASE (YQJM)

The monophasic and biphasic concurrent as well as the biphasic stepwise photo-chemo-enzymatic cascades have been in general performed as described above. The details about the reaction composition and reaction conditions applied for each cascade mode are displayed in Tab. S14.

Tab. S14. Details about photo-chemo-enzymatic reactions catalysed by EREDs.

Cascade mode	Reaction mode	Substrate(s)	Enzyme	Reaction composition	Reaction conditions for SAS oxidation	Reaction conditions for enzymatic step
concurrent	monophase one-pot		**ERED**	SAS (1 mM), 1.3 eq of NADH, 3.0 µM YqjM in 50 mM NaPi (pH 7.5), 10% (v/v) ACN, 20 mM substrate, V_{final} = 1 mL	O_2 atmosphere, T=30°C, λ>400 nm, 18-24 h	
	biphase one-pot			700 µL of SAS (1 mM), 1.3 eq of NADH, 3.0 µM YqjM dissolved in NaPi (50 mM, pH 7.5), 300 µL substrate, V_{final} = 1 mL		
stepwise	biphase two-pot		**ERED**	700 µL of 1 mM SAS in 50 mM NaPi (pH 7.5) was added to 300 µL substrate. The reaction was supplied with pure oxygen and light exposure and stirred at 200 rpm for 24 hours. The reaction mixture was then centrifuged and the organic phase was transferred to a new vial. To this solution, 1.3 eq of NADH, 3.0 µM YqjM in 50 mM NaPi (pH 7.5) were added.	O_2 atmosphere, T=30°C, λ>400 nm, 24 h	30°C, 18-24 h, 200 rpm

Exemplary time course analysis of reactions employing EREDs

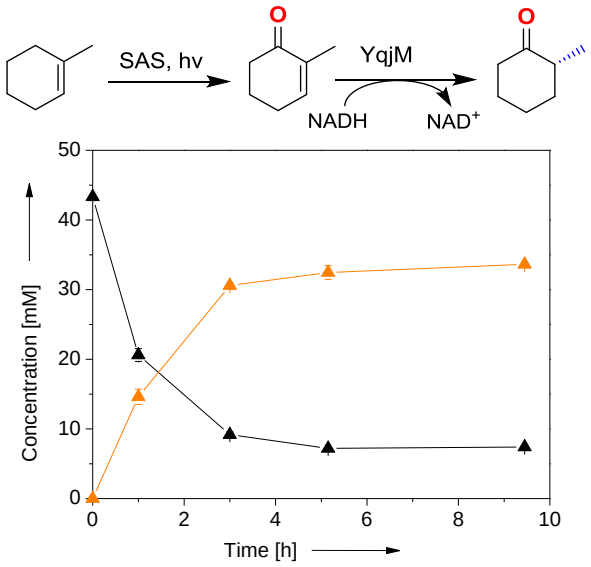


Fig. S13. Time course of the second step of the photo-chemo-enzymatic transformation for asymmetric reduction of C=C bonds in the step wise reaction mode: photooxidation of 1-methyl-1-cyclohexene to 2-methyl-2-cyclohexen-1-one (▲) using SAS and conversion to (*R*)-2-methylcyclohexanone (▲) catalysed by YqjM.

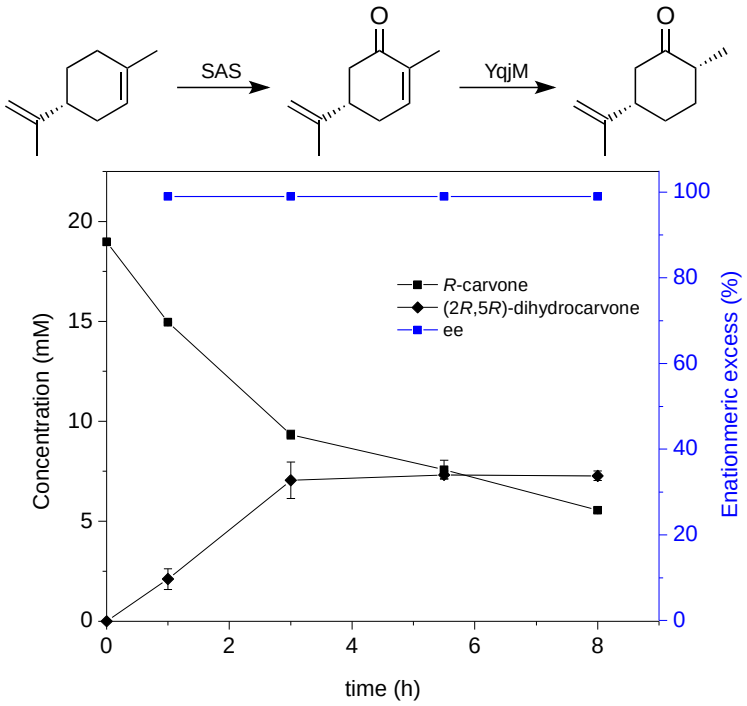


Fig. S14: Time course of the second step of the photo-chemo-enzymatic transformation reaction in the stepwise reaction mode starting from (*R*)-limonene.

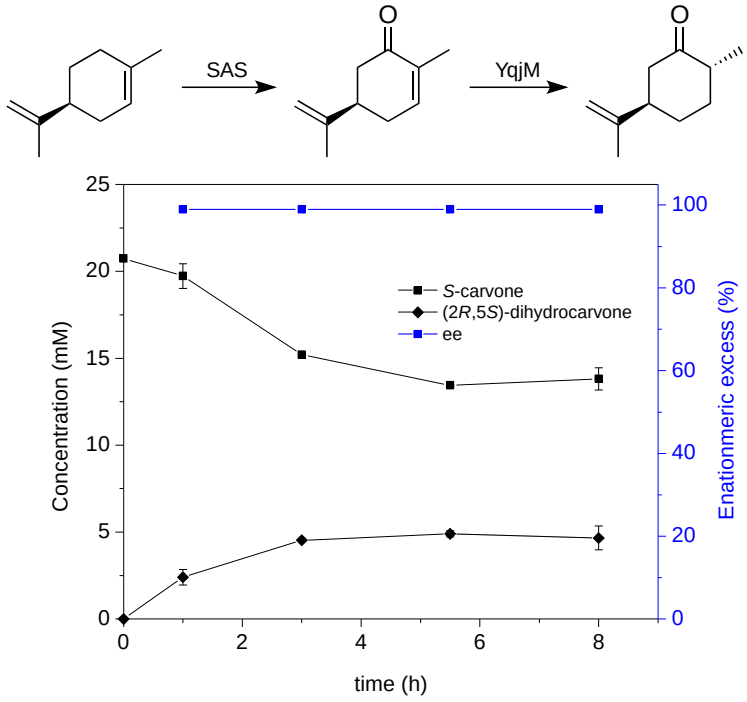
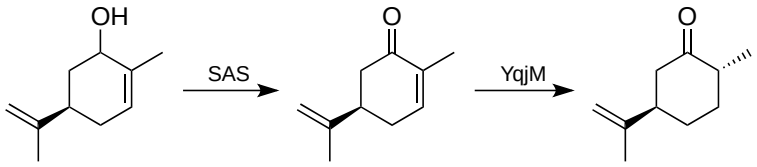


Fig. S15: Time course of the second step of the photo-chemo-enzymatic transformation reaction of ethylbenzene to 1-phenylethanol in a stepwise reaction mode starting from (*S*)-limonene

STEPWISE REACTIONS (MONOPHASE) STARTING FROM THE ALCOHOL

Tab. S15. Overview about the stepwise reactions starting directly from the alcohol.



Photooxidation using SAS, 30 h			**Ene-reduction using YqjM, 1h**		
	Conversion [%]	*Concentration of (R)-carvone [mM]*		*Conversion [%]*	*Concentration of (R)-dihydrocarvone [mM]*
No cosolvent	54.1	5.7	No cosolvent, without catalase	100	5.52
10% ACN	82.3	8.4	No cosolvent, with catalase	100	5.417
			10% ACN, without catalase	100	8.313
			10% ACN, with catalase	100	8.376

[a] Reaction conditions: 1 mM SAS and 10 mM (*R*)-carveol. In the second step, 1 µM YqjM, 1.3 eq NADH, 10 % (v/v) ACN, NaPi (50 mM) pH 7.5 were added.

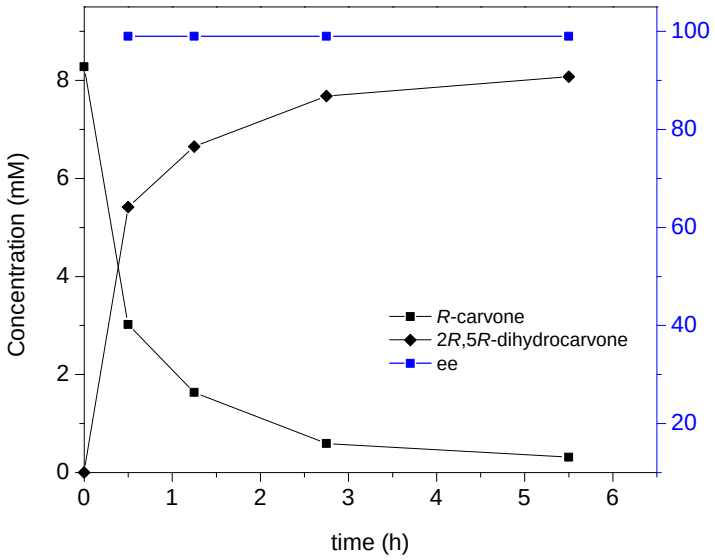
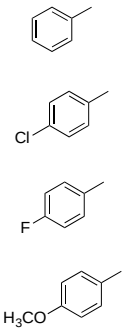


Fig. S16: Time course of the second step of the photo-chemo-enzymatic transformation reaction starting from carveol in a step-wise reaction mode.

7. ARYL ALCOHOL OXIDASE (AAO)

For AAO, only the biphasic stepwise photo-chemo-enzymatic cascades have been performed (as generally described above). The details about the reaction composition and reaction conditions applied for each cascade are displayed in Tab. S16.

Tab. S16. Details about photo-chemo-enzymatic reactions catalysed by AAO.

Cascade mode	Reaction mode	Substrate(s)	Enzyme	Reaction composition	Reaction conditions for SAS oxidation	Reaction conditions for enzymatic step
stepwise	biphase two-pot		**AAO**	700 µL of 1 mM SAS in 100 mM NaPi (pH 6.0) was added to 300 µL substrate. The reaction was supplied with pure oxygen and light exposure and stirred at 200 rpm for 24 hours. The reaction mixture was then centrifuged and the organic phase was transferred to a new vial. To this solution, 15 µM AAO in 100 mM NaPi (pH 6.0) were added.	O_2 atmosphere, T=30°C, λ>400 nm, 24 h	30°C, 18-24 h, 200 rpm

Time course analysis of reactions employing AAO

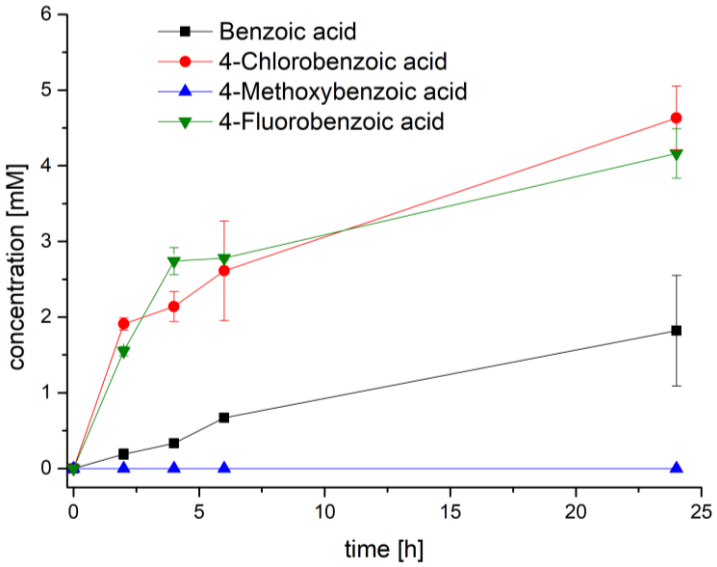


Fig. S17: Time course of the second step of the photo-chemo-enzymatic oxidation of toluene and derivatives in a step-wise reaction mode employing AAO for 22 hours.

PREPARATIVE SCALE SYNTHESES

a) Hydroxynitrile lyases (HNLs)

A mixture of 50 mL toluene and 50 mL of an aqueous phase containing 3 mM SAS in 50 mM citrate buffer pH 4.0 was stirred in a round bottomed flask provided with pure oxygen and light exposure at 30 °C. After 72 hours, the organic phase (43 mL) was transferred to an Erlenmeyer flask and 60 mL HCN 2 M (10 equivalents) and 1,5 mL *Pa*HNL were added. The reaction mixture was stirred at 200 rpm at room temperature for 22 h.

Fig. S18: Image of the obtained (*R*)-mandelonitrile from preparative scale synthesis.

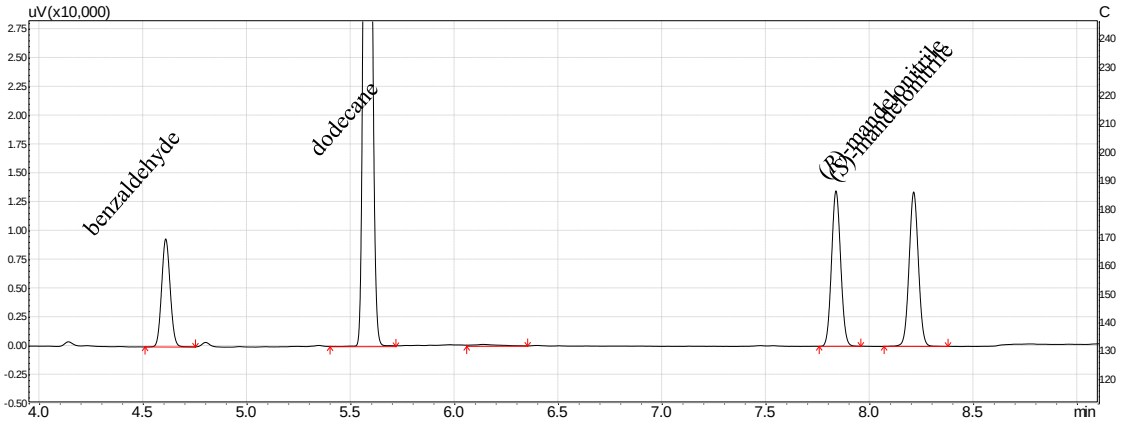
b) Benzaldehyde lyase (BAL)

A mixture of 50 mL toluene and 50 mL of an aqueous phase consisting of 3 mM SAS in 50 mM potassium phosphate buffer (pH 9.5) was stirred in a round bottomed flask provided with pure oxygen and light exposure at 30°C. After 24 hours, the organic phase (48 mL) was transferred to an Erlenmeyer flask containing a fresh aqueous phase. The fresh aqueous phase (48 mL) contained 0.5 mM thiaminpyrophosphate (TPP), 0.5 mM $MgCl_2$, 50 mg BAL lyophilisate in potassium phosphate buffer (50 mM, pH 9.5). The reaction mixture was stirred at 200 rpm at room temperature for further 24 h.

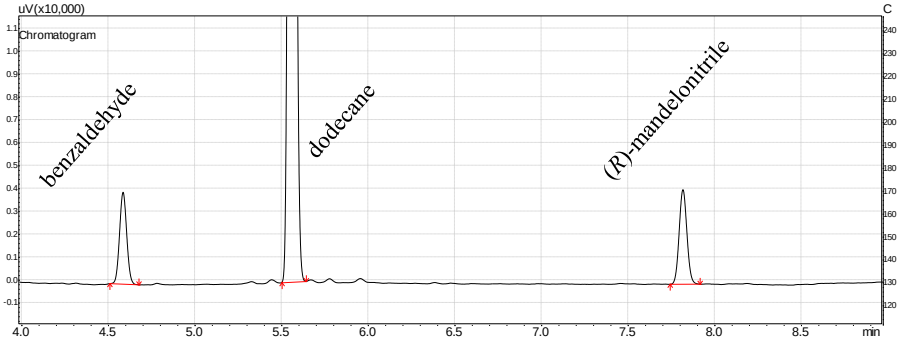
Fig. S19: Image of the obtained (*R*)-benzoin from preparative scale synthesis.

REPRESENTATIVE GC AND HPLC CHROMATOGRAMS

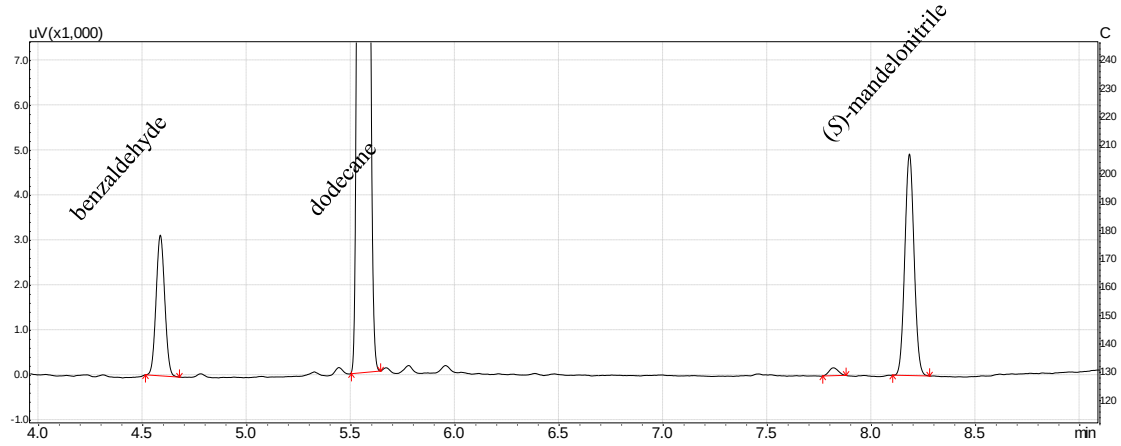
HYDROXYNITRILE LYASE REACTIONS (HNLs)



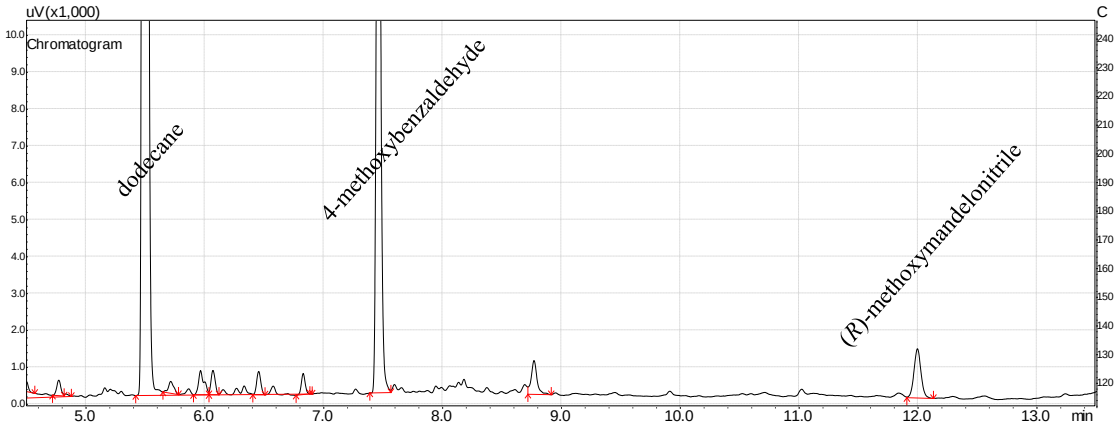
Representative GC chromatogram showing the baseline separation of (*R*)- and (*S*)- mandelonitrile. The dilution factor of the samples was 40.



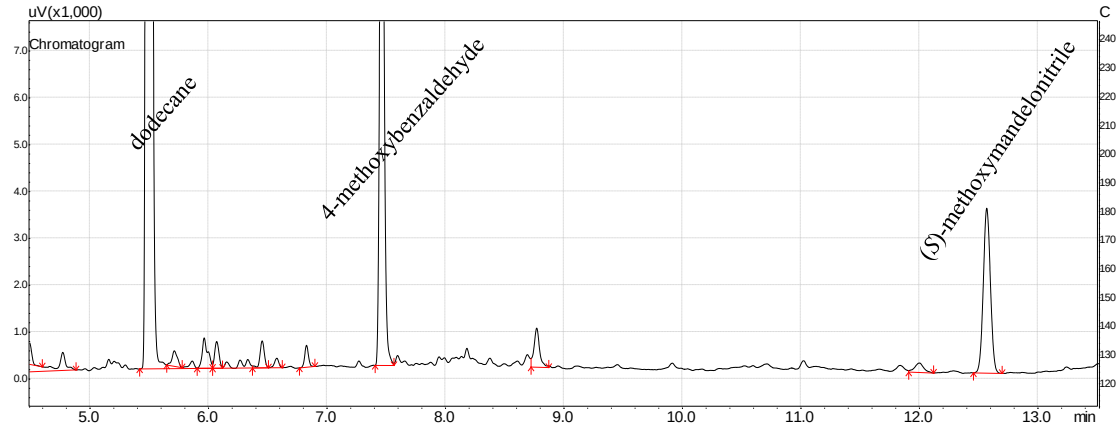
Representative GC chromatogram showing the conversion to (*R*)-mandelonitrile using (*R*)-selective HNL after 3.5 hour. The dilution factor of the samples was 40.



Representative GC chromatogram showing the conversion to (*S*)-mandelonitrile using (*S*)-selective HNL after 3.5 hour. The dilution factor of the samples was 40.

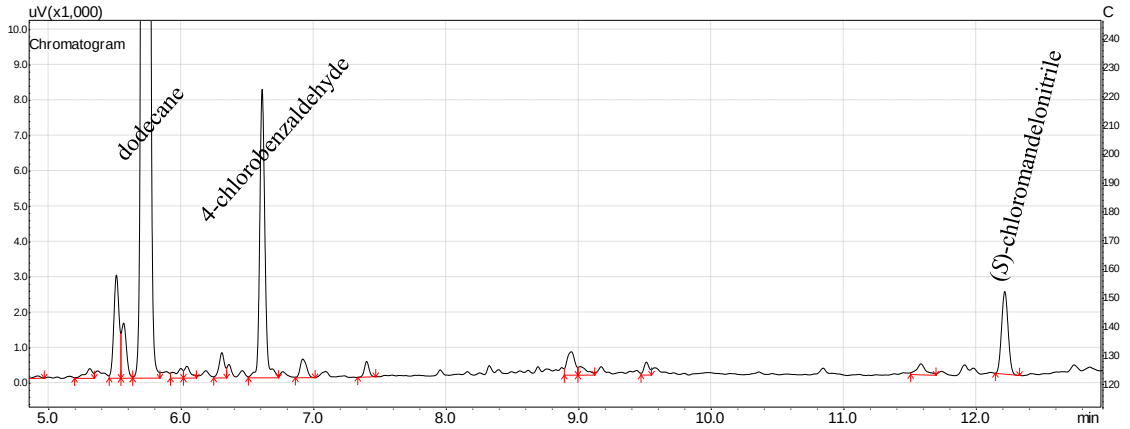


Representative GC chromatogram showing the conversion to (*R*)-methoxymandelonitrile using (*R*)-selective HNL after 3.5 hour. The dilution factor of the samples was 40.



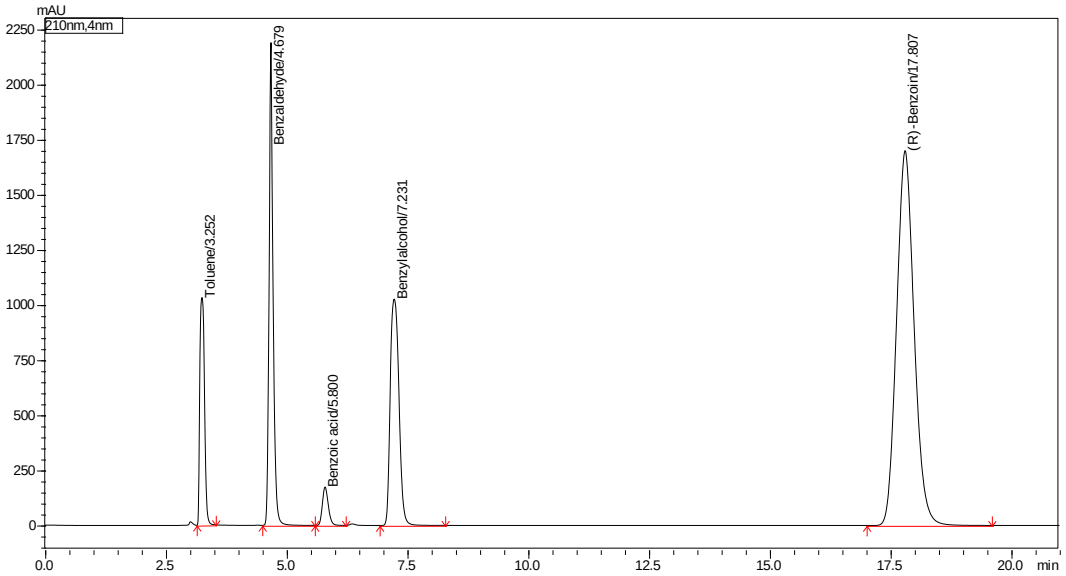
Representative GC chromatogram showing the conversion to (*S*)-methoxymandelonitrile using (*S*)-selective HNL after 3.5 hour. The dilution factor of the samples was 40.

Representative GC chromatogram showing the conversion to (*R*)-chloromandelonitrile using (*R*)-selective HNL after 3.5 hour. The dilution factor of the samples was 40.

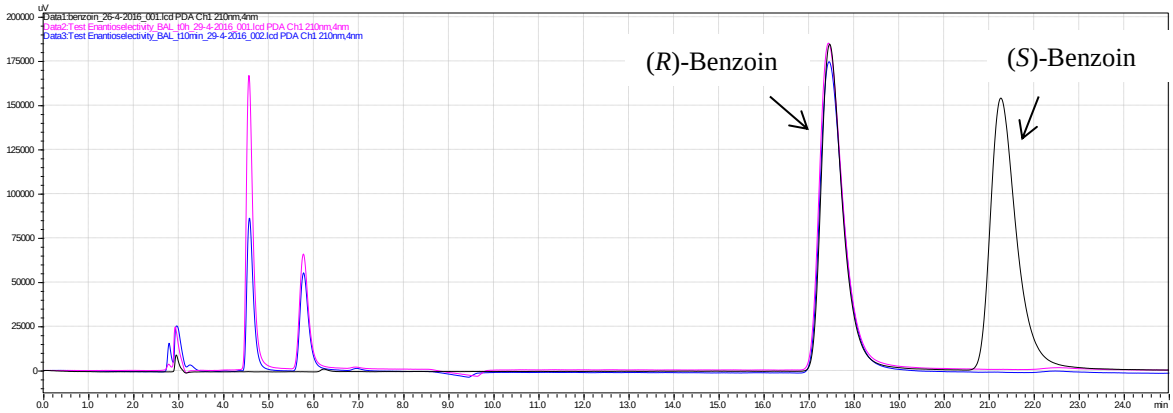


Representative GC chromatogram showing the conversion to (*S*)-chloromandelonitrile using (*S*)-selective HNL after 3.5 hour. The dilution factor of the samples was 40.

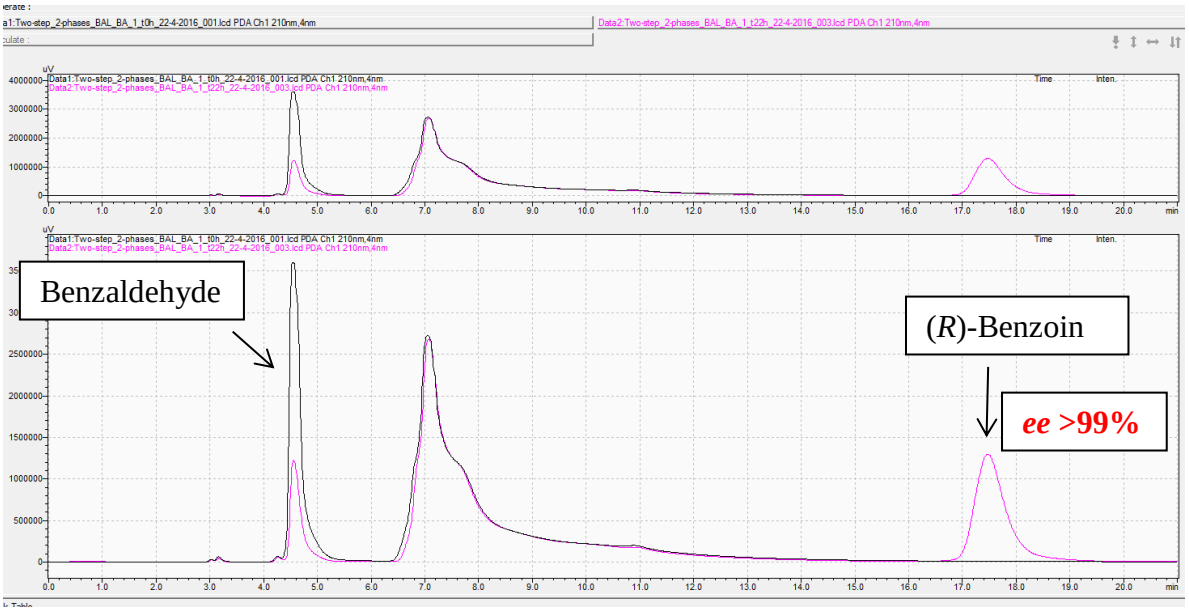
BENZALDEHYDE REACTIONS (BAL)



Representative HPLC-chromatogram showing all reaction components.

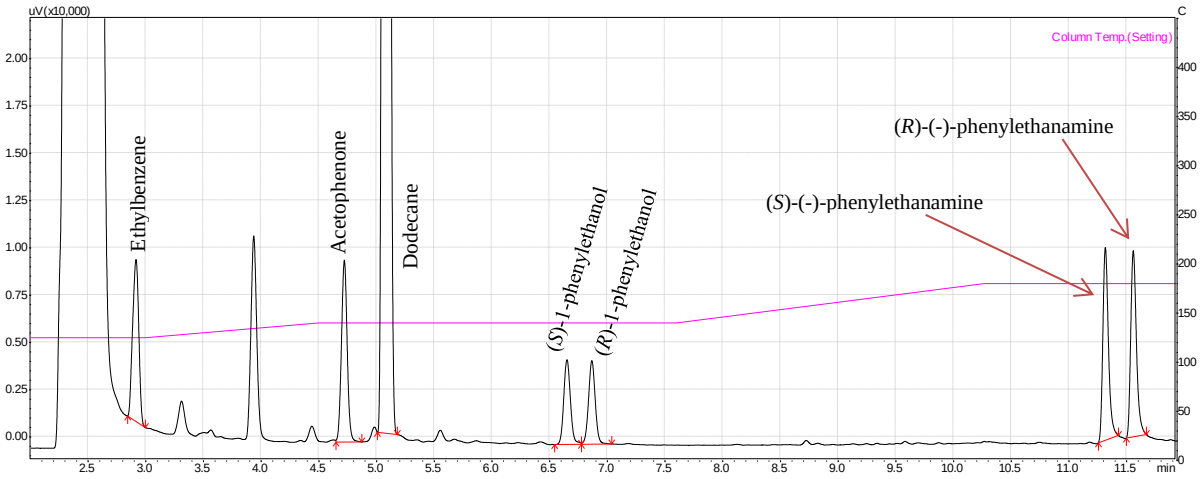


Representative HPLC chromatogram of the separation of (*S*)-and (*R*)-benzoin to determine the *ee.*

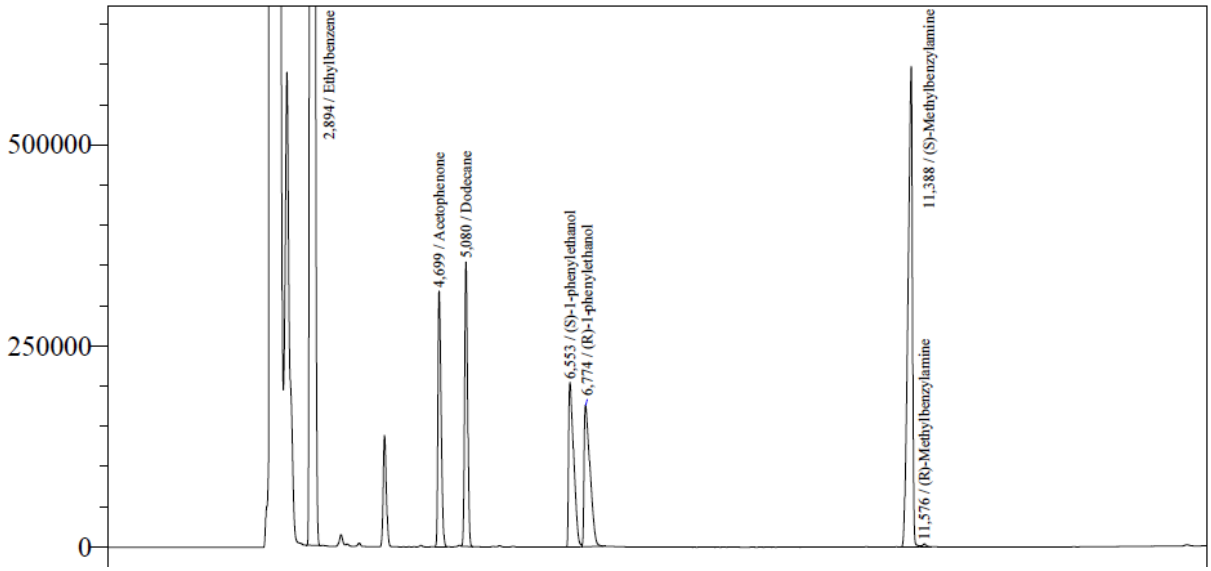


Time course of the two-step two-phases reaction starting from 80 mM benzaldehyde in the organic phase after 22 hours.

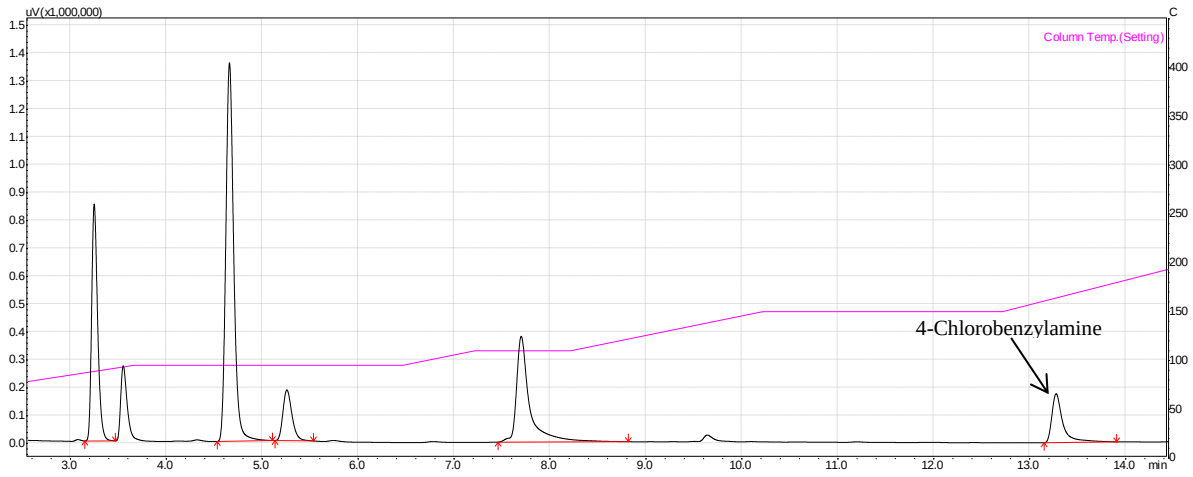
AMINE TRANSAMINASE REACTIONS (ATAS)



Representative GC chromatogram showing all reaction components and the separation of (*S*)- and (*R*)-phenylethanamine.

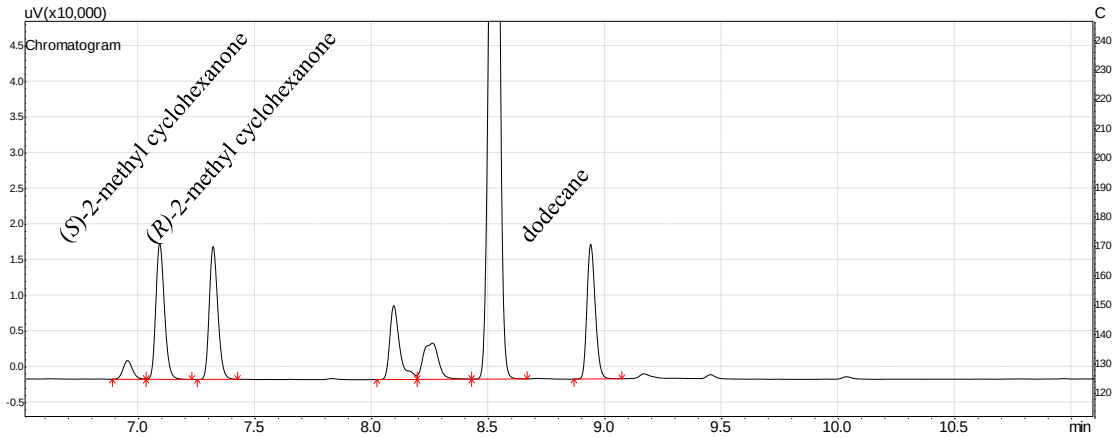


Representative GC chromatogram showing all reaction components and the separation of the formation of (*S*)-phenylethanamine from the (*S*)-ATA-catalysed reactions.

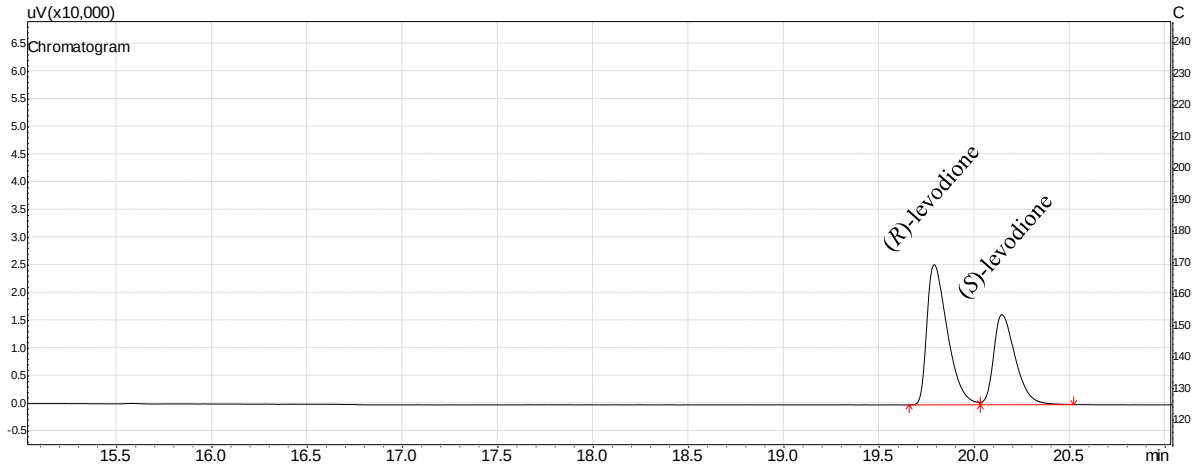


Representative GC chromatogram showing in the (*S*)-ATA-catalysed conversion of 4-Chlorotoluene to 4-Chlorobenzylamine after 1 hour. The dilution factor of the samples was 40.

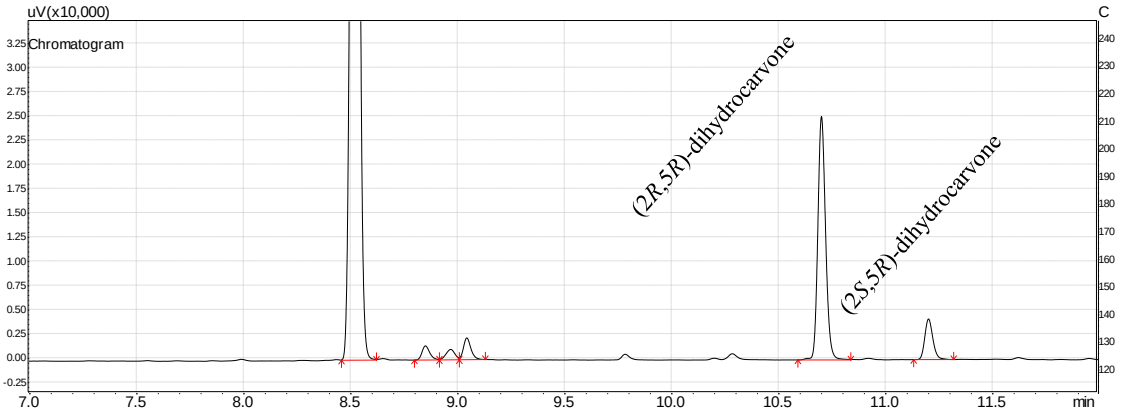
ENE-REDUCTASE (YQJM)



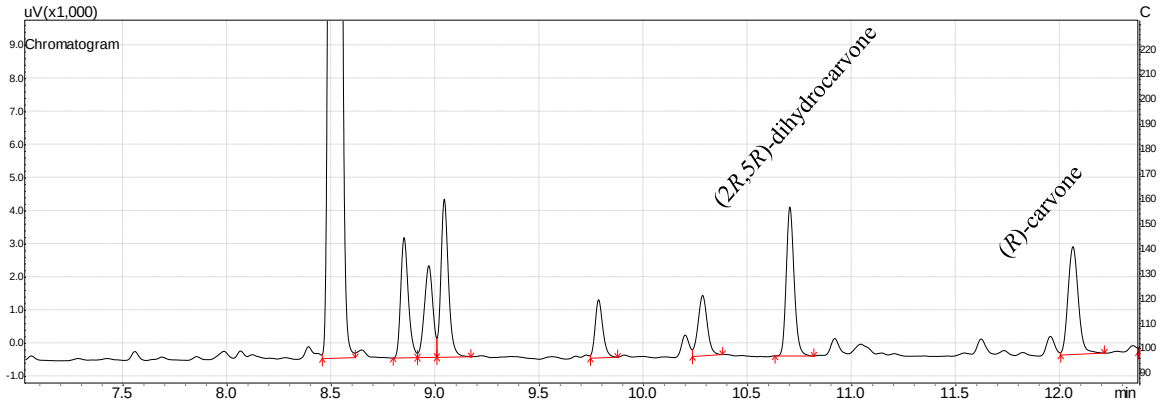
Representative GC chromatogram showing racemic 2-methylcyclohexanone. The dilution factor of the samples was 40.



Representative GC chromatogram showing the baseline separation of (*R*)-and (*S*)-levodione.

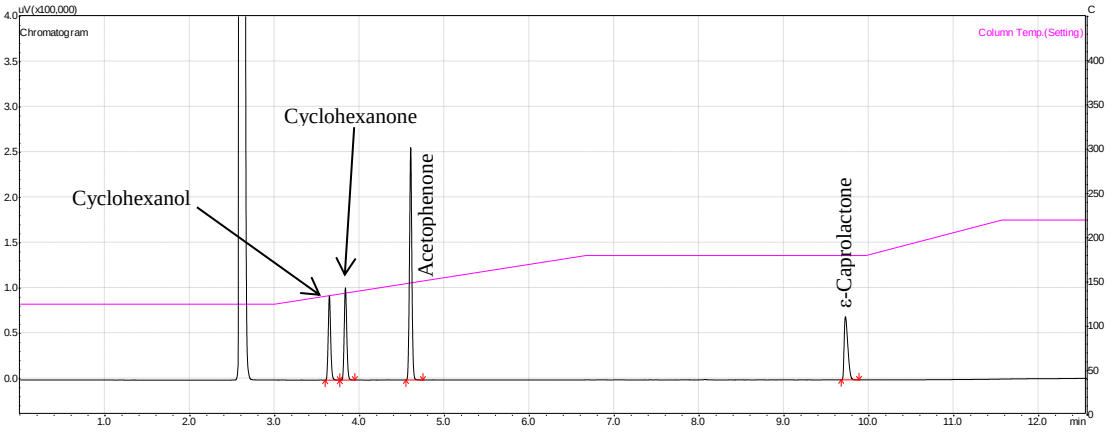


Representative GC chromatogram showing the baseline separation of (2*R*,5*R*)-and (2*S*,5*R*)-dihydrocarvone.

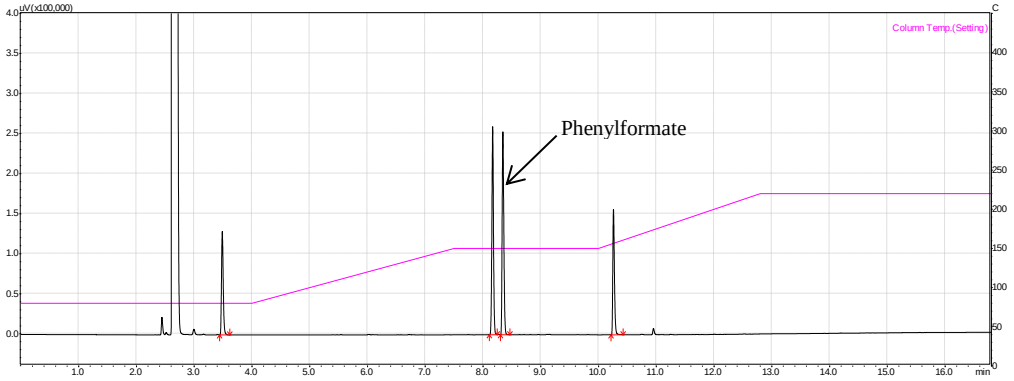


Representative GC chromatogram showing the formation of (2*R*,5*R*)-dihydrocarvone from (*R*)-carvone after 8h. The dilution factor of the samples was 40.

BAEYER-VILLIGER MONOOXYGENASES (BVMOS)

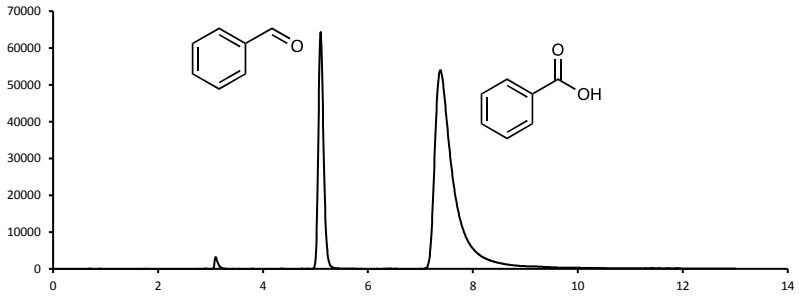


Representative GC chromatogram showing all reaction components.

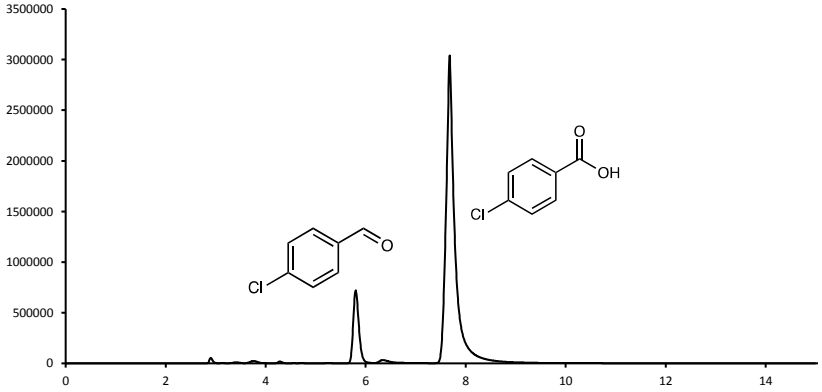


Representative GC chromatogram showing all reaction components.

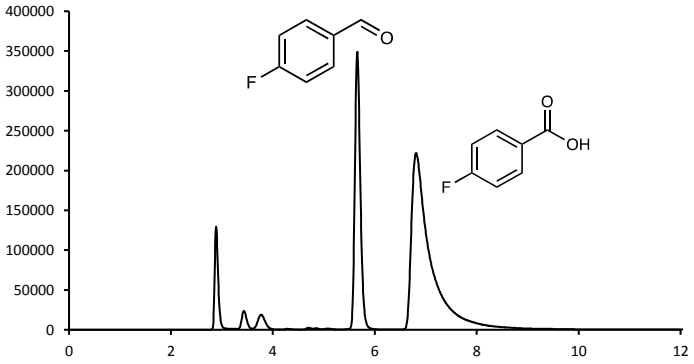
ARYL ALCOHOL OXIDASES (AAOS)



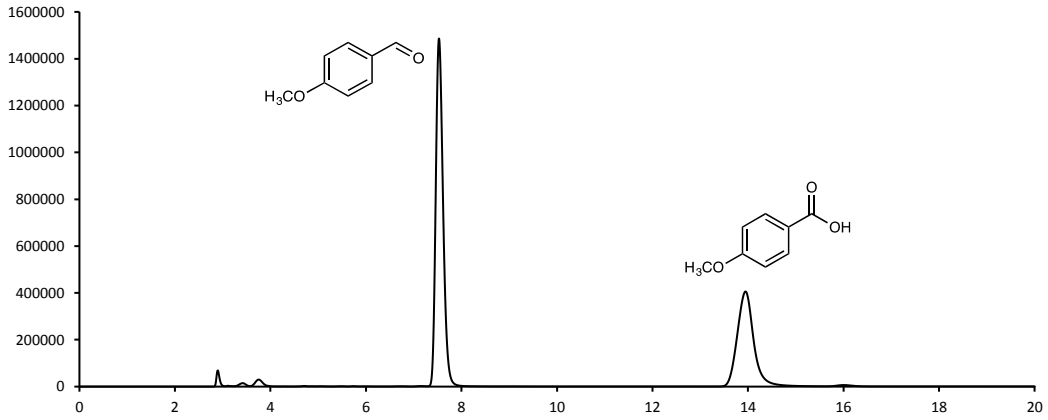
Representative chromatogram for the AAO-catalysed conversion of benzaldehyde to the corresponding benzoic acid. Method profile: 5 % B for 13 min (peak 1 (5.111): benzaldehyde and (peak 2 (7.110): benzoic acid. Solvent A: heptane; Solvent B: isopropanol with a flow of 1.0 mL/min. UV detection done at 215 nm.



Representative chromatogram for the AAO-catalysed conversion of 4-chlorobenzaldehyde to the corresponding 4-chlorobenzoic acid. Method profile: 5 % B for 10 min (peak 1 (5.798): benzaldehyde and (peak 2 (7.677): benzoic acid. Solvent A: heptane; Solvent B: isopropanol with a flow of 1.0 mL/min. UV detection done at 215 nm.



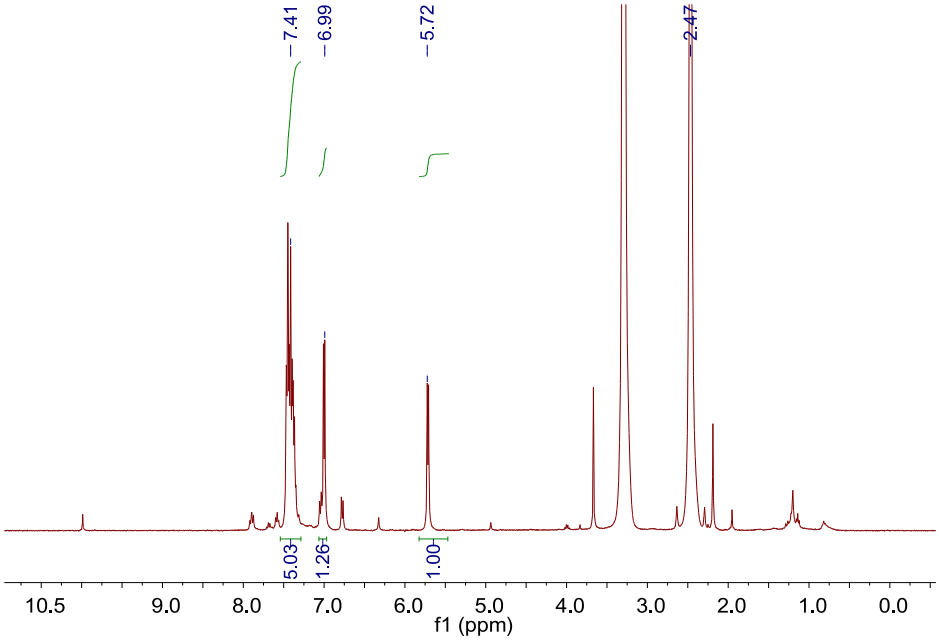
Representative chromatogram for the AAO-catalysed conversion of 4-fluorobenzaldehyde to the corresponding 4-fluorobenzoic acid. Method profile: 5 % B for 10 min (peak 1 (5.651): benzaldehyde and (peak 2 (7.571): benzoic acid. Solvent A: heptane; Solvent B: isopropanol with a flow of 1.0 mL/min. UV detection done at 215 nm.



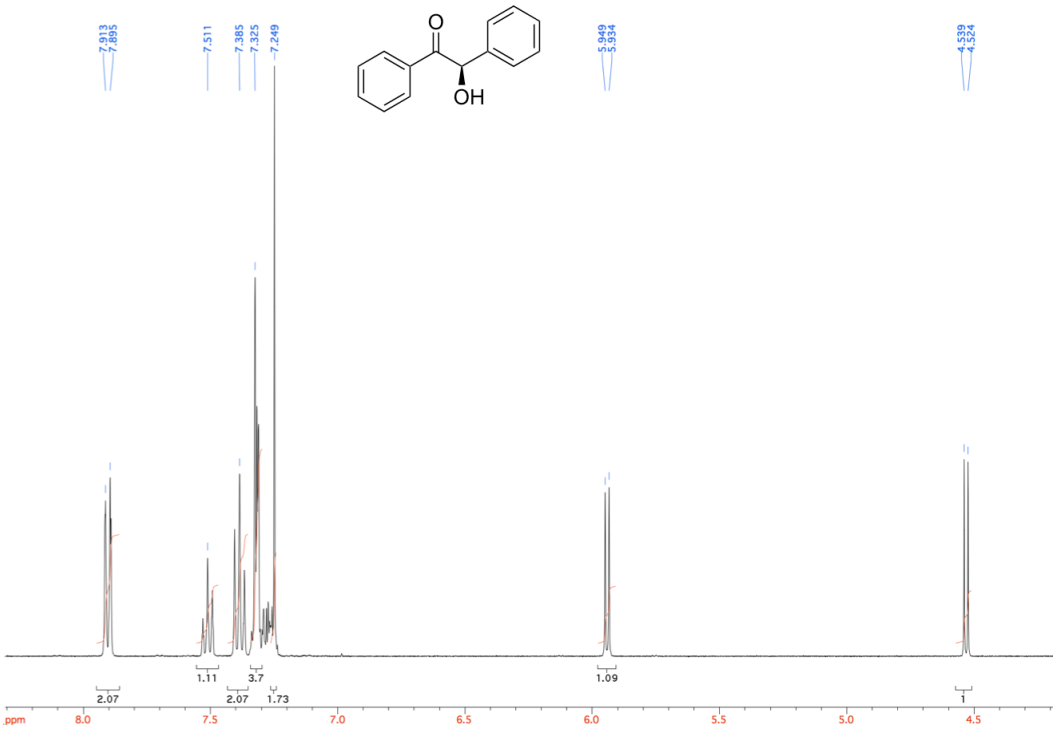
Representative chromatogram for the AAO-catalysed conversion of 4-methoxybenzaldehyde to the corresponding 4-methoxybenzoic acid. Method profile: 5 % B for 16 min (peak 1 (7.526): benzaldehyde and (peak 2 (13.945): benzoic acid. Solvent A: heptane; Solvent B: isopropanol with a flow of 1.0 mL/min. UV detection done at 215 nm.

REPRESENTATIVE NMR SPECTRA

PREPARATIVE SCALE SYNTHESES



^{1}H NMR in DMSO-d_6 from the product (*R*)-mandelonitrile extracted from the stepwise 2-phases preparative scale reaction after 22 hours (enzymatic step).

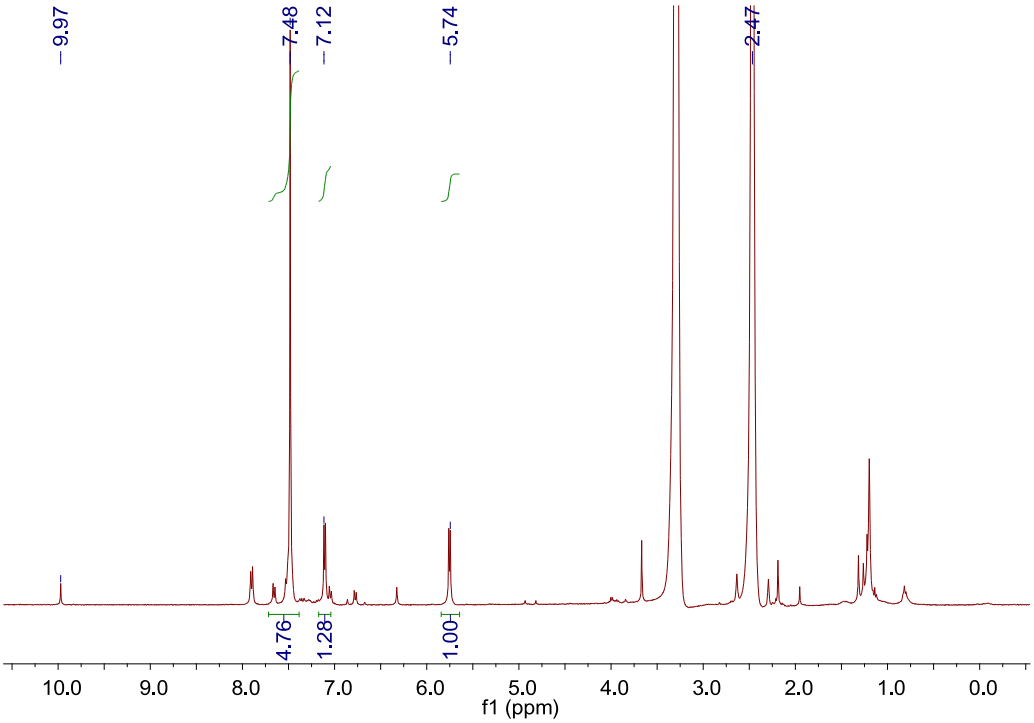


^{1}H NMR in $CDCl_3$ from the product (*R*)-Benzoin extracted from the stepwise 2-phases preparative scale reaction after 24 hours (enzymatic step).

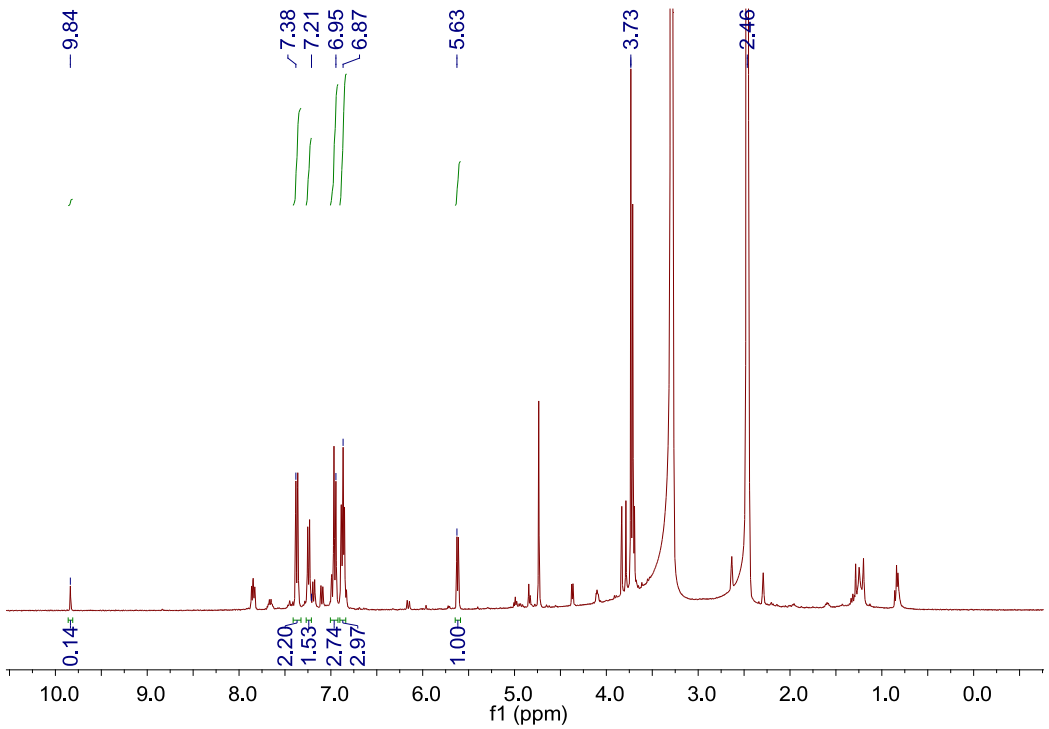
STEPWISE BIPHASIC REACTIONS

Note that the NMR spectra have been obtained from stepwise biphasic reactions after the enzymatic step was completed by taking a sample from the organic phase followed by evaporation of the organic phase (remaining substrate).

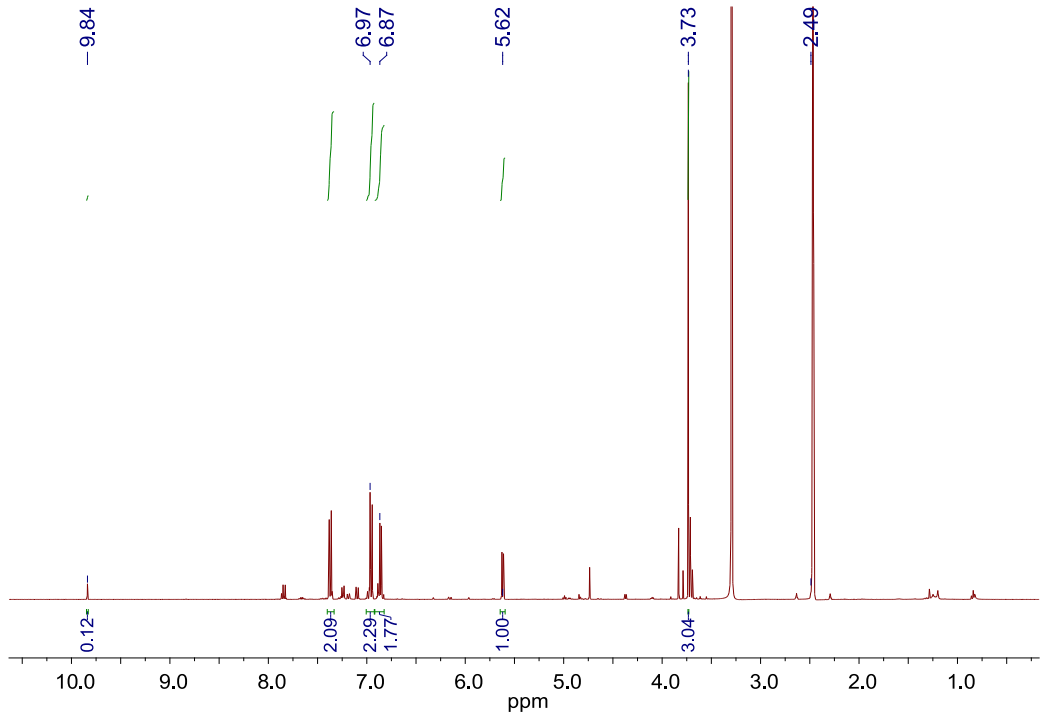
Hydroxynitrile lyase reactions (HNLs)



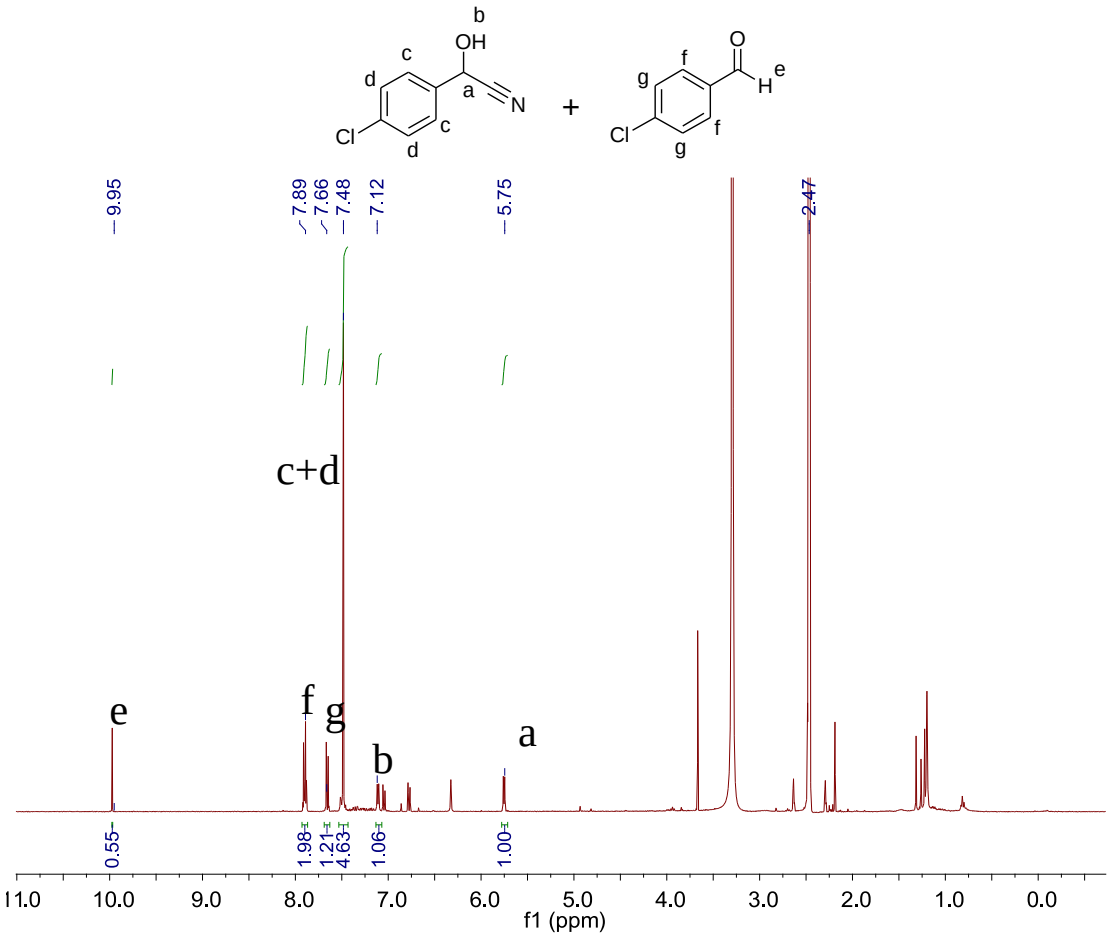
^{1}H NMR in DMSO-d_6 from the (*S*)- mandelonitrile product extracted from the stepwise 2-phases reaction after 24 hours.



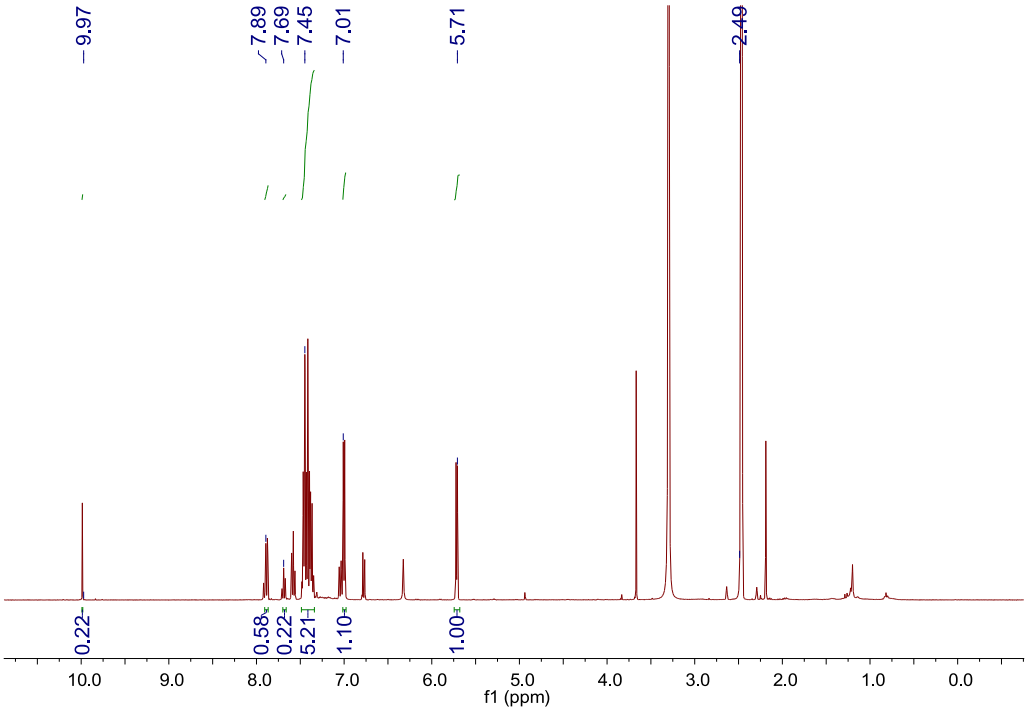
^{1}H NMR in DMSO-d_6 from the (*R*)-methoxymandelonitrile product extracted from the stepwise 2-phases reaction after 24 hours.



^{1}H NMR in DMSO-d_6 from the (*S*)-methoxymandelonitrile product extracted from the stepwise 2-phases reaction after 24 hours.

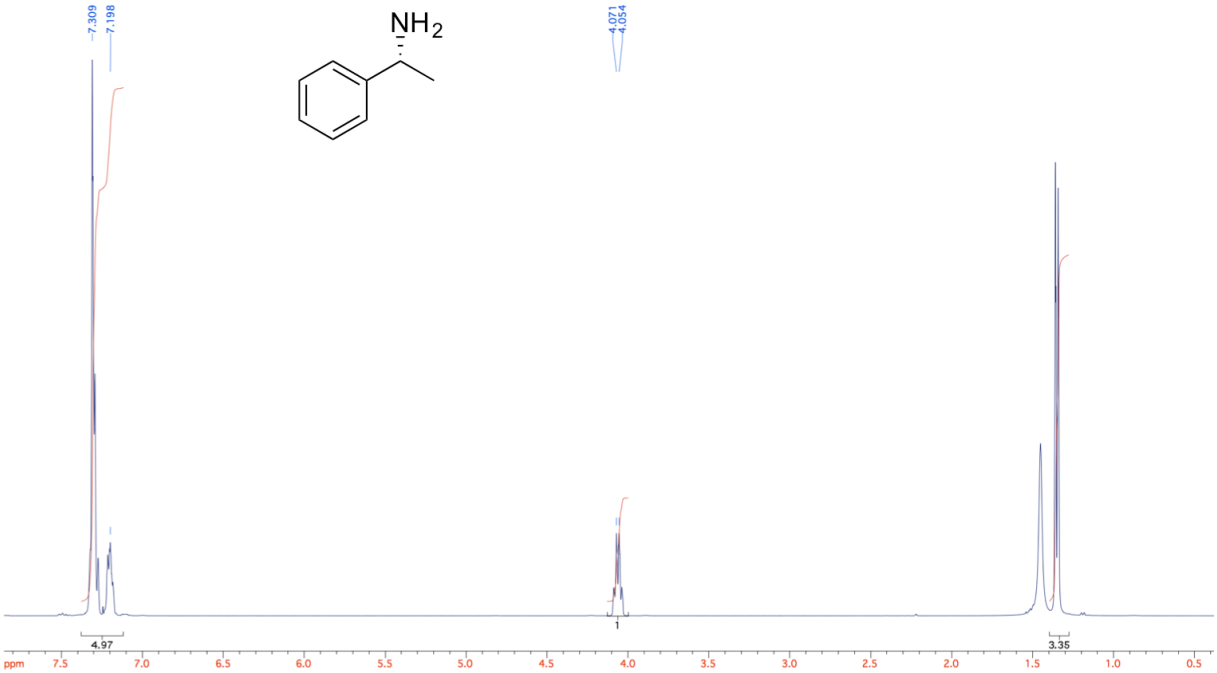


^{1}H NMR in DMSO-d_6 from the (*R*)-chloromandelonitrile product extracted from the stepwise 2-phases reaction after 24 hours.

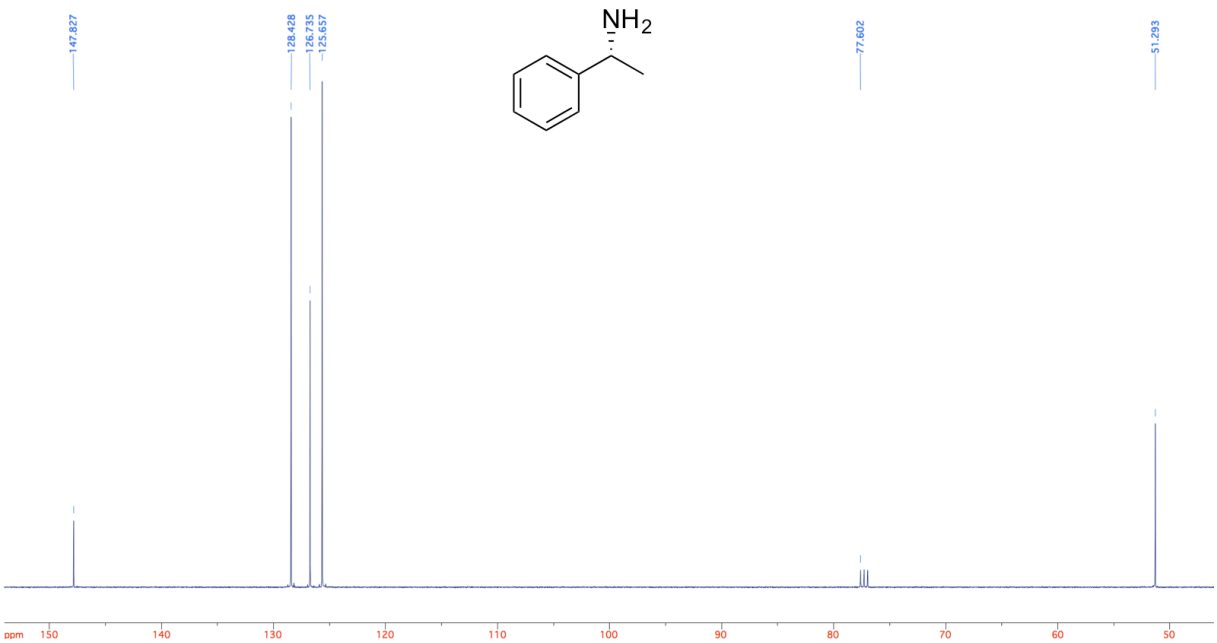


^{1}H NMR in DMSO-d_6 from the (*S*)-chloromandelonitrile product extracted from the stepwise 2-phases reaction after 24 hours.

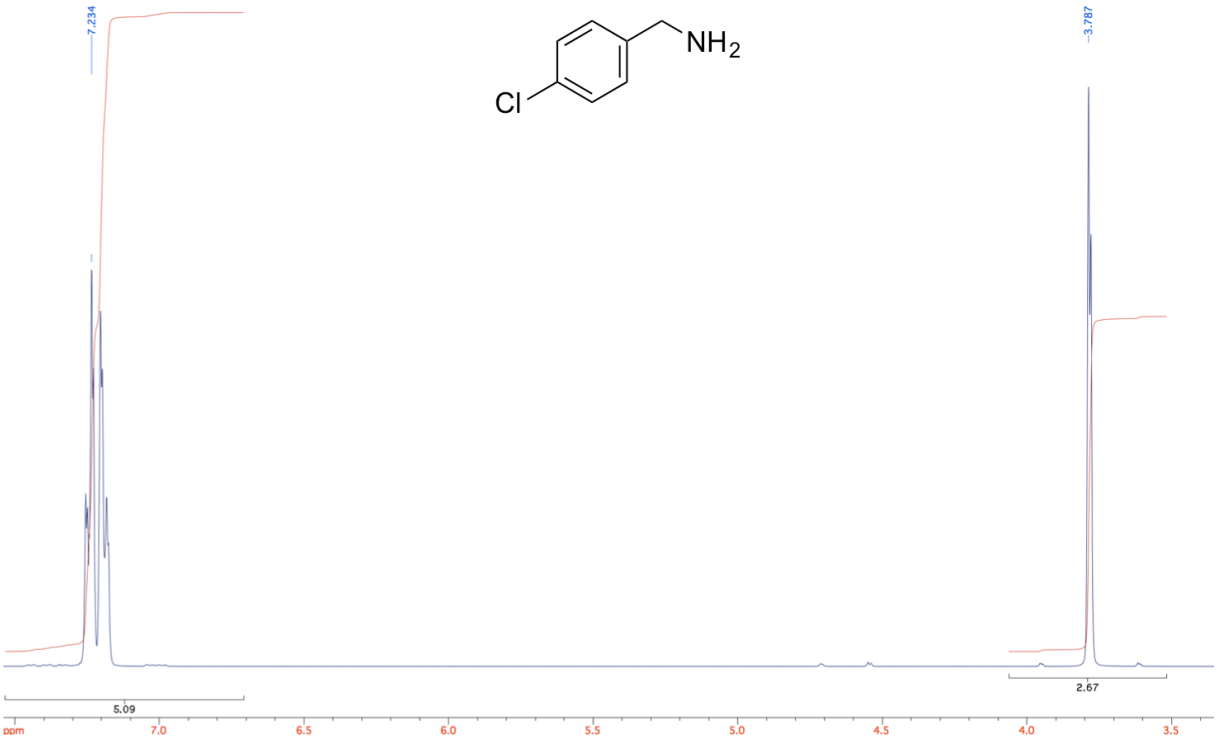
Amine transaminase reactions (ATAs)



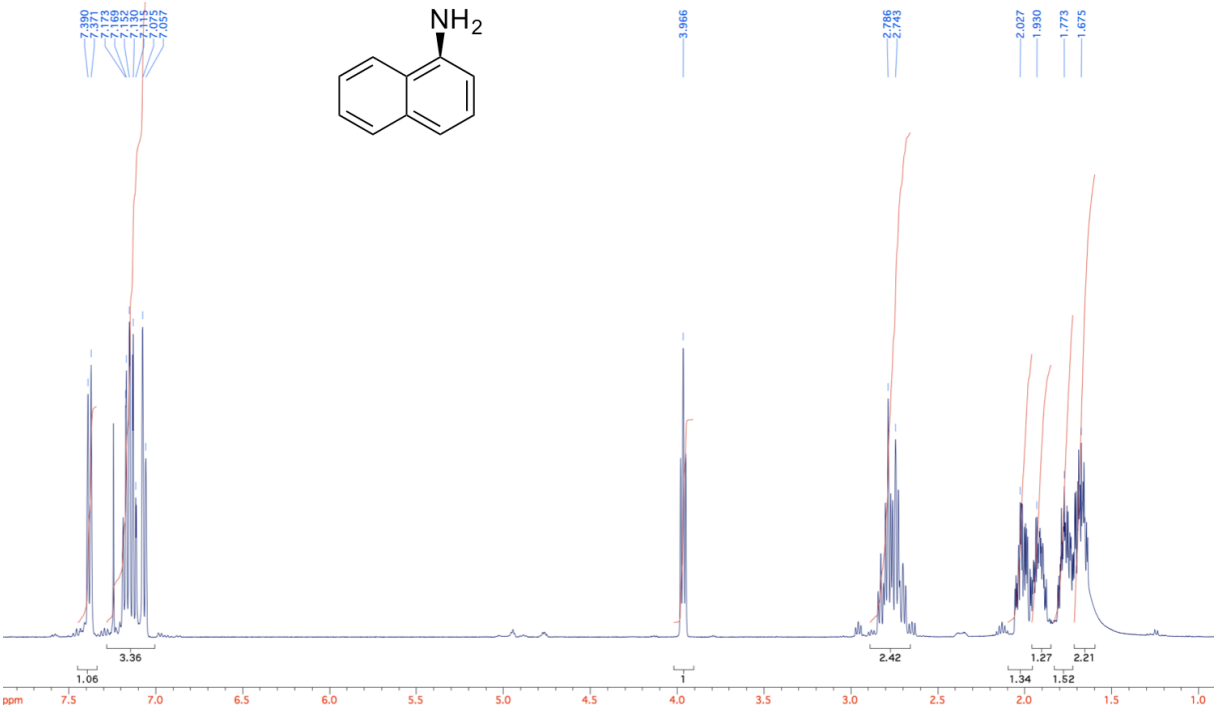
^{1}H NMR analysis in $CDCl_3$ of (*S*)-(-)-phenylethanamine obtained from the reaction after 28 hours and evaporation of the organic phase.



^{13}C NMR analysis in $CDCl_3$ of (*S*)-(-)-phenylethanamine obtained from the reaction after 28 hours and evaporation of the organic phase. The signal at around 77 ppm corresponds to $CDCl_3$.

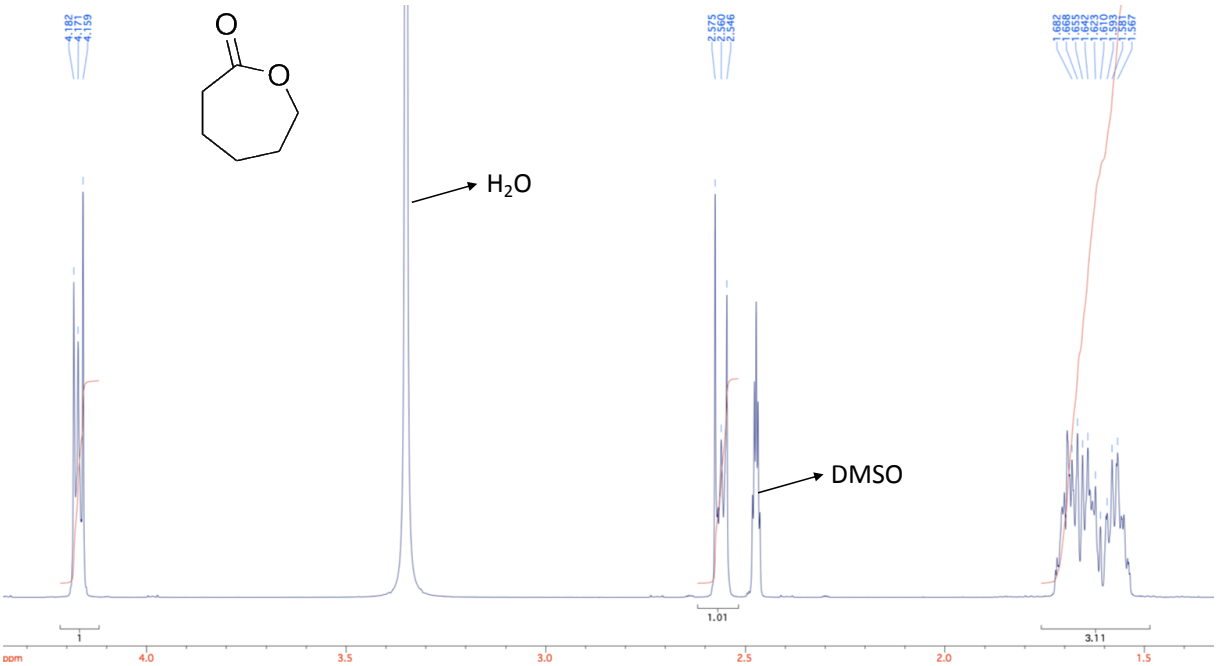


^{1}H NMR analysis in $CDCl_3$ of 4-chlorobenzylamine obtained from the reaction after 28 hours and evaporation of the organic phase.

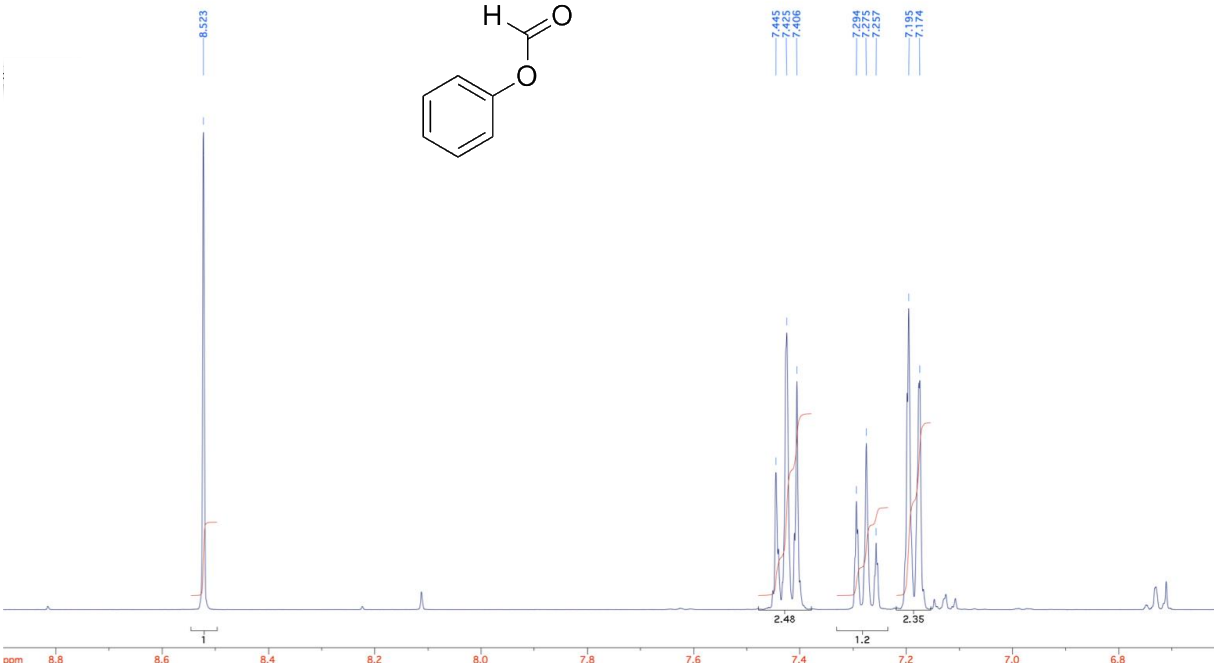


^{1}H NMR analysis in $CDCl_3$ of (*R*)-(-)-1,2,3,4-tetrahydro-1-naphthylamine obtained from the reaction after 22.5 hours and evaporation of the organic phase.

Baeyer-Villiger monooxygenases (BVMOs)



^{1}H NMR analysis in dmso-d_6 of ε-caprolactone obtained from the reaction after 20 hours and evaporation of the organic phase.



^{1}H NMR analysis in dmso-d_6 of phenylformate obtained from the reaction after 22 hours and evaporation of the organic phase.

REFERENCES

[1] H. Man, K. Kedziora, J. Kulig, A. Frank, I. Lavandera, V. Gotor-Fernandez, D. Rother, S. Hart, J. P. Turkenburg, G. Grogan, *Top. Catal.* **2014**, *57*, 356-365.

[2] a) A. Weckbecker, W. Hummel, *Biocatal. Biotransform.* **2006**, *24*, 380-389; b) C. Rodriguez, W. Borzecka, J. H. Sattler, W. Kroutil, I. Lavandera, V. Gotor, *Org. Biomol. Chem.* **2014**, *12*, 673-681.

[3] F. J. Ruiz-Duenas, P. Ferreira, M. J. Martinez, A. T. Martinez, *Protein Expression Purif.* **2006**, *45*, 191-199.