# SUPPLEMENTARY INFORMATION:

# One-pot hydroaminomethylation of an alkene under formation of primary amines by combining hydroformylation at elevated syngas pressure and biocatalytic transamination in water

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## Contents

# 1 Analytics

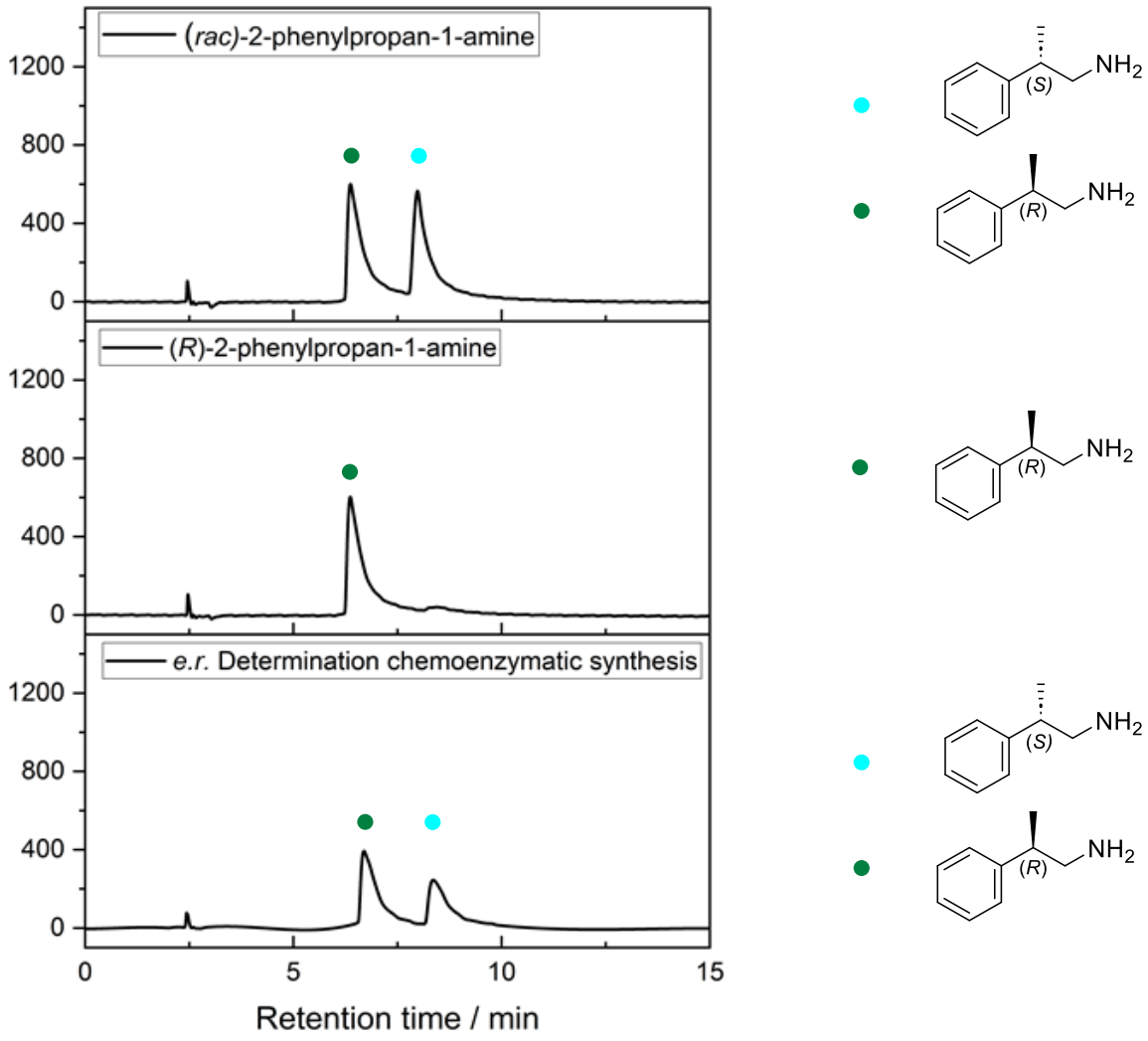
## 1.1 High-performance liquid chromatography

High-pressure liquid chromatography (HPLC) was carried out on the Azura HPLC device from Knauer. A chiral Chiralpak® AD-H column (particle size 5 µm, 250 mm x 46 mm) was applied utilizing *n*-hexane (95.5%, v/v) containing trifluoroethanoic acid (0.5%, v/v) as mobile phase and isopropanol (4.5%, v/v) as eluent. An isocratic flow (1.3 mL/min) was applied at a constant back pressure of ~64 bar at 30 °C for 15 min. The absorbance was measured at 210 nm and 254 nm.

**DAD detector**: time constant 0.1 s, sampling rate 10 hz, autozero at start, bandwith 8 mm.

**Injection volume:** 10 µL.

**Running time:** 15 min.




**S 1:** HPLC chromatograms including the ones for the reference compounds and a representative chromatogram for the determination of the *e.r.* of a reaction product resulting from the chemoenzymatic synthesis. The measurement was conducted with an UV-detector at 210 nm. The substances were applied at a total concentration of 1 mg/ml. Retention time: 6.1 (*R*)-2-phenylpropan-1-amine, 8.1 min (*S*)-2-phenylpropan-1-amine.

### 1.2 Gas chromatography

Gas chromatography (GC) measurements were performed on Shimadzu Nexis GC-2030 with a Phenomenex Zebron ZB-5MSi non-chiral column (0.25 mm ID, 0.20 µm film thickness and 30 m length) and FID detector.

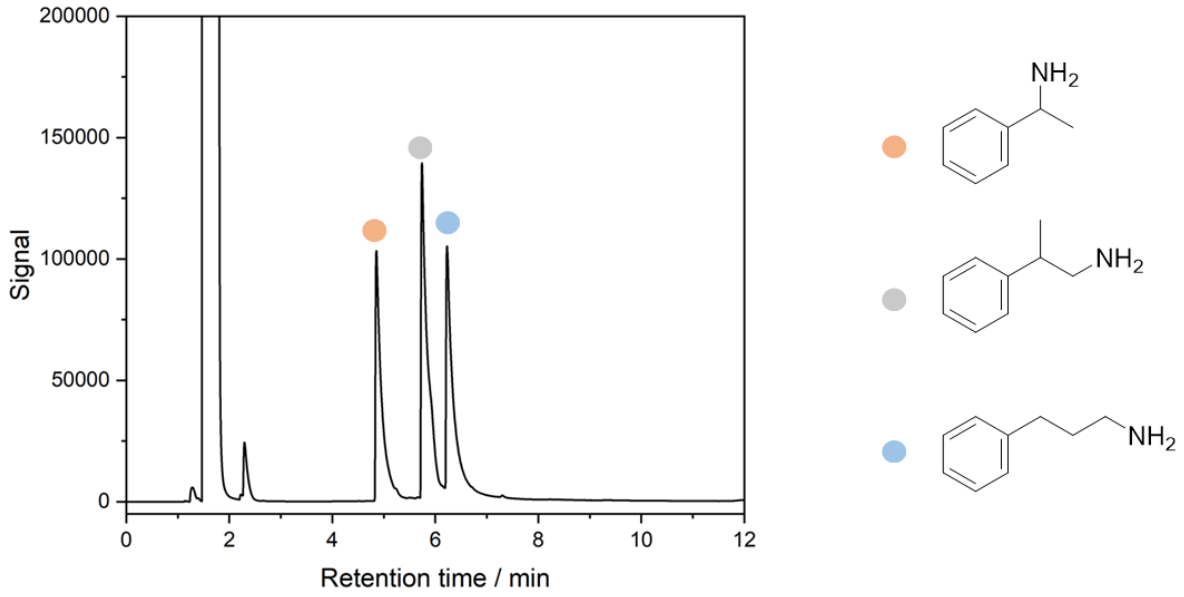
**Settings split**: 200 °C, carrier gas: $N_2$, pressure: 138.6 kPa, total flow: 29.0 mL/min, column flow: 2.36 mL/min, linear velocity: 46.9 cm/s, purge flow: 3.0 mL/min, split ratio: 10.0.

**Settings oven:** 35 °C for 1 min, 35 to 110 °C with 20°C/min, to 130 °C with 10 °C/min with 5 min hold time, to 200 °C with 60 °C/min.

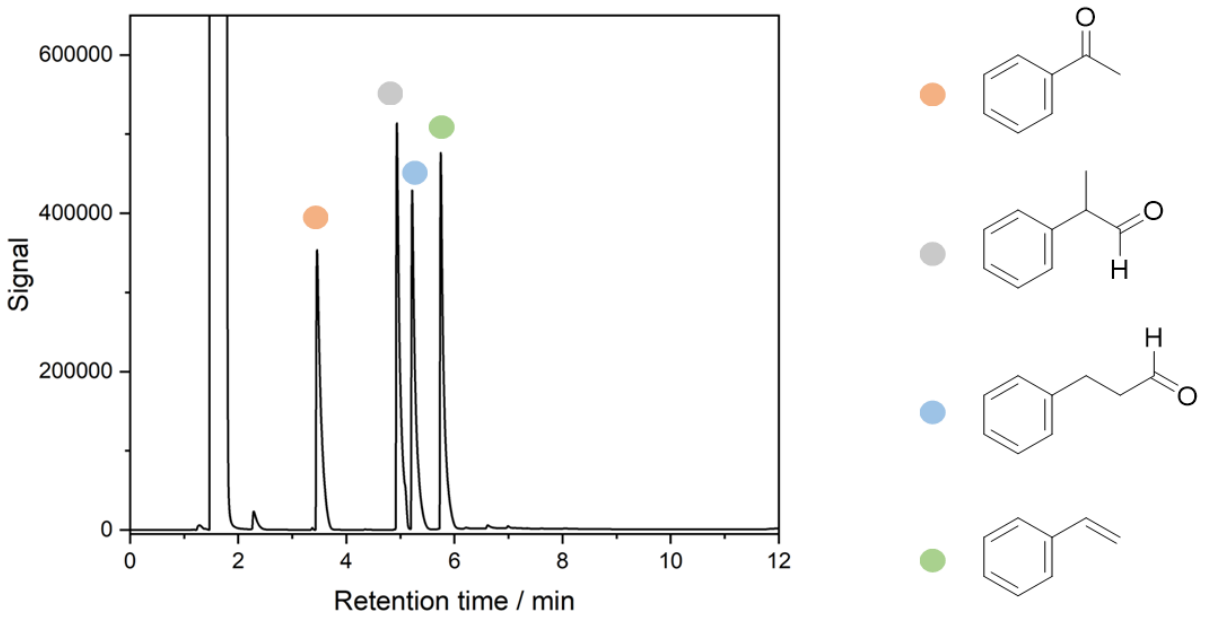
**Settings FID:** 230 °C, sampling rate: 40 ms, make up flow ($N_2$): 40 mL/min, $H_2$ flow: 40 mL/min, air flow: 400 mL/min.

**Injection volume:** 1.0 µL.

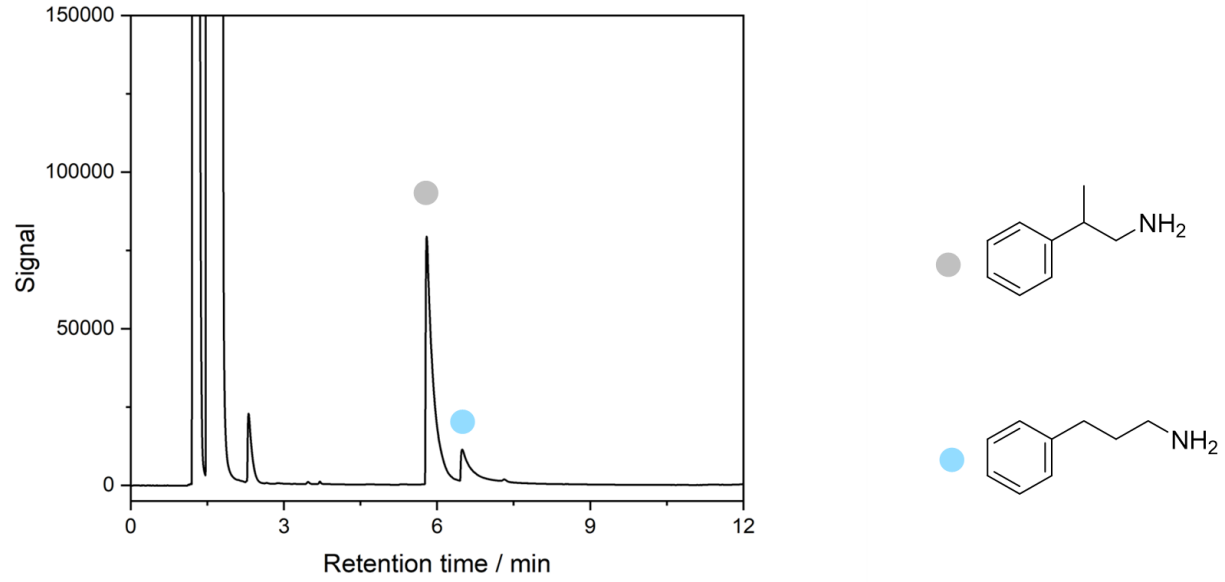
**Running time:** 12 min.




**S 2:** Exemplary GC chromatogram. The signal was measured with an FID detector, and references were applied at a total concentration of 0.5 mg/ml.
Retention time: 4.8 min 1-phenylethylamine, 5.7 min 2-phenylpropan-1-amine, 6.1 min 3-phenylpropan-1-amine.




**S 3:** Exemplary GC chromatogram. The signal was measured with an FID detector, and references were applied at a total concentration of 0.5 mg/ml.
Retention time: 3.6 min styrene, 5.2 min acetophenone, 5.4 min *rac*-2-phenylpropanal, 5.9 min 3-phenylpropanal.




**S 4**: Exemplary GC chromatogram of the crude products received from chemoenzymatic synthesis of primary amines starting from styrene including the products 2-phenylpropane-1-amine and 3-phenylpropan-1-amine.
Retention time: 5.7 min 2-phenylpropan-1-amine, 6.3 min 3-phenylpropan-1-amine.

### 1.3 Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance (NMR) spectra were recorded at room temperature on a Bruker Avance 500HD. The chemical shifts ($\delta$) were reported in parts per million (ppm) s = singlet, d = doublet, t = triplet). All spectra were referenced to the NMR-solvent $CDCl_3$ with a chemical shift of 7.26 ppm.

### 1.4 Determination of conversion, enantiomeric ratio and maximum error

The conversion was measured *via* GC in triplicate or fivefold determination and is defined as follows:

$$GC\ yield = "Conversion" = \frac{Amount\ of\ product\ obtained}{Theoretical\ amount\ of\ product} \cdot 100\%$$

The maximum error was determined by subtracting the highest deviation $x_j$ from the mean $\tilde{x}$ and is defined as follows:

$$maximum\ error = |x_i - \bar{x}|$$

The enantiomeric ratio (*e.r.*) was determined by measuring the peak areas of enantiomer 1 ($m_1$) and enantiomer 2 ($m_2$) *via* chiral HPLC. The *e.r.* is defined as follows:

$$enantiomeric\ ratio = e.r. = \left(\frac{m_1}{m_1 + m_2} \cdot 100\%\right) : \left(\frac{m_2}{m_1 + m_2} \cdot 100\%\right)$$

# 2 Heterologous protein expression

## 2.1 Transformation of *Escherichia coli* BL21-(DE3)

Plasmid DNA (~5 ng) was added to the chemically competent *Escherichia coli (E. coli)* BL21-(DE3) (50 µL aliquot) cells. The cells were incubated on ice for 30 min. Next, the cells were heat shocked (42 °C, 90 s) and stored on ice for 10 min. Subsequently, the cells were mixed with LB medium (700 µL) and incubated for ~1 h at 37 °C and 800 rpm. On an LB agar plate with the respective antibiotics, the cells were plated and incubated at 37 °C overnight.

## 2.2 Overnight cultures

Under sterile conditions, a sterile culture tube with a vented cap containing LB-medium (5 mL) and the corresponding antibiotics was inoculated with a single transformed *E. coli* BL21-(DE3) colony. The culture tube was incubated overnight at 180 rpm and 37 °C.

## 2.3 Heterologous protein expression

Initially, an overnight culture of transformed *E. coli* BL21-(DE3) was prepared. TB medium (1 L) in 5 L Erlenmeyer flasks (without baffles) was supplemented with the appropriate antibiotics. The TB medium was inoculated with the overnight culture at a 1:100 dilution and incubated at 37 °C with shaking at 180 rpm until an OD600 of 0.6-0.8 was reached. IPTG was then added to a final concentration of 1 mM. The main culture was incubated at 20 °C with shaking at 180 rpm for 20 h. After 20 h, the cultures were centrifuged at 4 °C and 4000x g for 30 min, the supernatants were discarded, and the cell pellets were stored at -20°C.

## 2.4 Cell disruption

The cell pellet was resuspended in potassium phosphate buffer (PPB, 250 mM, pH 7) at a pellet-to-buffer ratio of 1:2 (w/w). Under ice bath cooling, the cells were lysed *via* ultrasound, with three cycles of 3 min sonication at 20% power. Each cycle was followed by a 5 min cooling period. Subsequently, the lysate was centrifuged at 4 °C and 21000x g for 10 min. The supernatant (crude extract) was separated and filtrated (0.2 µm) and a sterile crude extract aliquot was stored at -20 °C.

# 3 Qualification of protein overexpression

## 3.1 Bradford assay

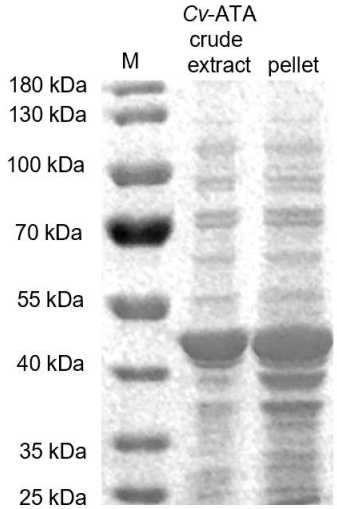
A 96-well microtiter plate was utilized for the Bradford assay. In each well, 5 µL of the diluted sample (1:25, 1:50, or 1:100), the buffer or a standard BSA series was prepared. Subsequently, 250 µL of Bradford reagent was added to each well, and the microtiter plate was incubated with shaking at 25 °C for 15 min. The absorbance was measured at 595 nm using the Multiskan GO® from Thermo Fisher Scientific.
All measurements were carried out in triplicates. The absorbance of the PPB buffer (250 mM, pH 7) was subtracted from the absorbances of the standard BSA series or the samples. A linear regression was calculated to determine the protein concentrations (mg/mL) of the sample (Equation 1).

Parameters:
slope of the linear fit (ΔA/Δt), buffer absorbance ($A_{buffer}$), sample absorbance ($A_{probe}$).

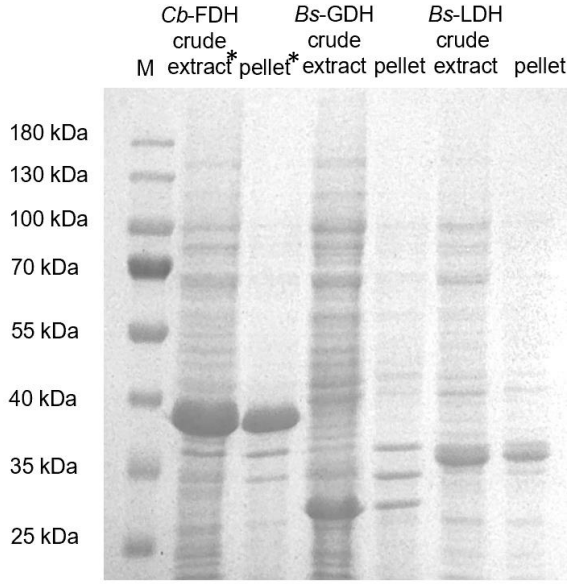
$$c_{probe} = \frac{A_{probe} - A_{buffer}}{\frac{\Delta A}{\Delta t}} \quad (1)$$

## 3.2 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Initially, the overall protein concentration of the samples was determined *via* the Bradford assay. The Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was conducted at a protein concentration of 1 µg/µL of a respective sample. Each sample was prepared in a total volume of 30 µL. The 6x Laemmli buffer (6 µL) and $ddH_2O$ were used for dilution. Subsequently, the sample was incubated at 95 °C (10 min) and centrifuged (4000x g, 1 min).
The PageRuler Prestained Protein Ladder from Thermo Fisher Scientific (5 µL) was utilized as a marker. The remaining wells of the SDS gel (12% resolving gel, 5% stacking gel) were filled with 5 µL of the marker and with 5 µL of each sample. Initially, the electrophoresis was conducted at 12.5 mA per gel for 30 min. The current strength was increased to 25 mA per gel for a total running time of approximately 2 h. After electrophoresis, the stacking gel was discarded, and the resolving gel was washed with $dH_2O$. Subsequently, the gel was incubated in α-cyclodextrin staining solution for approximately 1 h. The gel was washed with $dH_2O$ and stored in $dH_2O$ overnight. Further decolorization of the gel was unnecessary when utilizing this method.




**S 5:** SDS-PAGE analysis of *Chromobacterium violeceum* aminotransferase (*Cv*-ATA). The crude extract and cell pellet were applied with a protein concentration of 1 µg/µL (5 µL sample). The PageRuler Prestained Protein Ladder from Thermo Fisher Scientific was used as a molecular weight marker.




**S 6:** SDS-PAGE analysis of *bacillus subtilis* glucose dehydrogenase (*Bs*-GDH) or *bacillus subtilis* lactate dehydrogenase (*Bs*-LDH). The crude extract and cell pellet were applied with a protein concentration of 1 µg/µL (5 µL sample). The PageRuler Prestained Protein Ladder from Thermo Fisher Scientific was used as a molecular weight marker.

*Moreover, the formate dehydrogenase from *Candida boidinii* (*Cb*-FDH) was overexpressed and assayed in the SDS-PAGE. However, for this research project it was not further used.

# 4 Optical assays

## 4.1 Optical activity assays

The activity assays of NADH/NAD+ dependent enzymes were carried out in 96-well microtiter plate format at the Spark 10M microtiter plate spectrometer device from Tecan. Each assay was performed in a sixfold determination. The assays were carried out in a potassium phosphate buffer (PPB, 100 mM, pH 7), with a total reaction volume of 250 µL. The absorbance was measured at 340 nm for 60 s. The volumetric activity of each enzyme-containing crude extract was expressed as units/mL (U/mL).

*Bs*-GDH:

A 157 mM D-glucose solution (238 µL) in PPB (100 mM, pH 7) was prepared resulting in a final D-glucose concentration of 150 mM. The *Bs*-GDH crude extract (2 µL, dilution factor 1:40) was added, and the assay was initiated by the addition of 10 µL of a 10 mM $NAD^+$ stock solution in potassium phosphate buffer (100 mM, pH 7), resulting in a final $NAD^+$ concentration of 0.4 mM.

*Bs*-LDH:

A 10.5 mM pyruvate solution (238 µL) in PPB (100 mM, pH 7) was prepared resulting in a final pyruvate concentration of 10 mM. The *Bs*-LDH crude extract (2 µL, dilution factor 1:10) was added, and the assay was initiated by the addition of 10 µL of a 10 mM NADH stock solution in potassium phosphate buffer (100 mM, pH 7), resulting in a final NADH concentration of 0.4 mM.

The volumetric activities of the enzymes were calculated as follows. The slope ΔA/Δt was obtained by linear regression, and the volumetric activity of the enzyme was calculated by equation 2.

$$V\text{olumetric activtiy} \,/\, \mathrm{U \cdot mL^{-1}} = \frac{\frac{\Delta A}{\Delta t} \cdot V_T \cdot \mathrm{F}}{\varepsilon \cdot \mathrm{d} \cdot V_E}$$

(2)

Parameters:
$V_t$ = total volume (250 µL), F = dilution factor, ε = extinction factor (0.662 mL · µmol$^{-1}$ · mm$^{-1}$), d = layer thickness of 8 mm, VE = 2 µL diluted enzyme crude extract.

**Table 1:** Volumetric activity of *Bs*-GDH and *Bs*-LDH was obtained *via* an optical assay.

| Biocatalyst | Volumetric activity without additives |
|---|---|
| *Bs*-GDH | 331 U/mL |
| *Bs*-LDH | 524 U/mL |

**Table 2:** Additives for optical assays of *Cb*-FDH, *Bs*-GDH or *Bs*-LDH. The [Rh(acac)$(CO)_2$] and 6-DPPon concentrations are expressed as mol% relative to a 50 mM substrate concentration.

| | **Additive concentration** |
|---|---|
| Additive 1 | 0.1 wt%, 0.2 wt% or 0.4% Triton X-100 |
| Additive 2 | 5% (v/v), 10% (v/v), or 15% (v/v) DMSO |
| Additive 3 | 0.34 mol% [Rh(acac)$(CO)_2$], 1.8 mol% 6-DPPon additive with 10% (v/v) DMSO;<br>0.68 mol% [Rh(acac)$(CO)_2$], 3.6 mol% 6-DPPon additive with 10% (v/v) DMSO;<br>1.36 mol% [Rh(acac)$(CO)_2$], 7.2 mol% 6-DPPon additive with 10% (v/v) DMSO |
| Additive 4 | 0.34 mol% [Rh(acac)$(CO)_2$], 1.8 mol% 6-DPPon additive with 0.2 wt% Triton X-100;<br>0.68 mol% [Rh(acac)$(CO)_2$], 3.6 mol% 6-DPPon additive with 0.2 wt% Triton X-100;<br>1.36 mol% [Rh(acac)$(CO)_2$], 7.2 mol% 6-DPPon additive with 0.2 wt% Triton X-100 |

## 4.2 Optical activity assays of transaminase

*Cv*-ATA

The optical transaminase assay was conducted in a 96-well UV-plate format in a sixfold determination using the Spark 10M microtiter plate spectrometer device from Tecan. The assay was conducted in potassium phosphate buffer (PPB, 100 mM, pH 7), with a total reaction volume of 114 µL. The absorbance was measured at 245 nm for 60 s.

The reaction mixture (104 µL) contained 11.4 mM (*S*)-1-phenylethylamine (final concentration 10 mM) and 2 µL of crude extract in PPB (100 mM, pH 7). The assay was started by adding 10 µL of PPB (100 mM, pH 7) containing 114 mM pyruvate (final pyruvate concentration 1 mM). The volumetric activity of the crude extract was expressed as units/mL (U/mL).

The volumetric activity of transaminases was calculated as follows. The slope ΔE/Δt was obtained by linear regression, and the volumetric activity of the enzyme was calculated using Equation 2.

Parameters:
$V_t$ = total volume (114 µL), F = dilution factor, ε = extinction factor (0.0012 mL · µmol$^{-1}$ · mm$^{-1}$), d = layer thickness of 3.6 mm, $V_E$ = 2 µL enzyme crude extract.

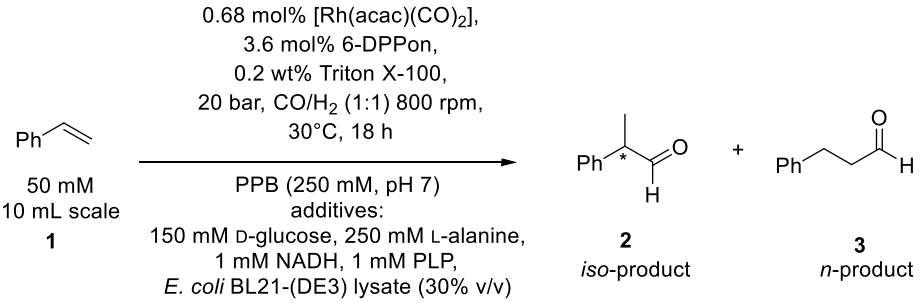
**Table 3:** Volumetric activity of Cv-ATA was measured using an optical activity assay.

| Biocatalyst | Volumetric activity |
|---|---|
| *Cv*-ATA | 23 U/mL |

**Table 4:** Additives for optical assay of *Cv*-TA. The [Rh(acac)(CO)$_2$] and 6-DPPon concentrations are expressed as mol% relative to a 50 mM substrate concentration.

| | Additive concentration |
|---|---|
| Additive 1 | 0.1 wt%, 0.2 wt% or 0.4% Triton X-100 |
| Additive 2 | 5% (v/v), 10% (v/v), or 15% (v/v) DMSO |
| Additive 3 | 0.34 mol% [Rh(acac)(CO)$_2$], 1.8 mol% 6-DPPon additive with 10% (v/v) DMSO;<br>0.68 mol% [Rh(acac)(CO)$_2$], 3.6 mol% 6-DPPon additive with 10% (v/v) DMSO;<br>1.36 mol% [Rh(acac)(CO)$_2$], 7.2 mol% 6-DPPon additive with 10% (v/v) DMSO |
| Additive 4 | 0.34 mol% [Rh(acac)(CO)$_2$], 1.8 mol% 6-DPPon additive with 0.2 wt% Triton X-100;<br>0.68 mol% [Rh(acac)(CO)$_2$], 3.6 mol% 6-DPPon additive with 0.2 wt% Triton X-100;<br>1.36 mol% [Rh(acac)(CO)$_2$], 7.2 mol% 6-DPPon additive with 0.2 wt% Triton X-100 |

## 5 Hydroformylation




**S 7:** Hydroformylation of styrene (**1**) utilizing the 6-DPPon ligand and [Rh(acac)(CO)$_2$] catalyst. The 6-DPPon/Rh-catalyzed hydroformylation of styrene (**1**) was conducted in an aqueous biphasic system mediated by the surfactant Triton X-100, with the following additives: 250 mM L-alanine, 150 mM D-glucose and ammonium formate, 1 mM NADH and PLP, and sterile crude extract of empty vector *E. coli* BL21-(DE3) (30%, v/v).

**Lab safety: CAUTION: Syngas is a very hazardous gas and must be handled with all necessary safety precautions!**

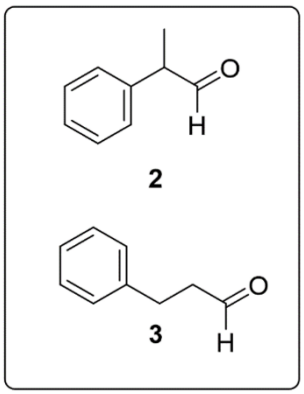
**Procedure:** Hydroformylation of styrene (**1**) was conducted in triplicate utilizing autoclaves (75 mL from Parr, Series 5000 Multiple Reactor System with 75 mL vessels from Parr Instrument Company) with glass inlets under inert conditions at a total reaction volume of 10 mL.

Initially, the [Rh(acac)(CO)$_2$] (0.9 mg, 3.4 µmol, 0.68 mol%), 6-DPPon (5.0 mg, 18 µmol, 3.6 mol%), and Triton X-100 (20 mg 0.2 wt%) were weighed directly into the glass inlet. The autoclave was sealed, vacuumed, and purged with argon (3x) with the glass inlet inserted. Under an argon atmosphere, degassed potassium phosphate buffer (PPB, 250 mM, pH 7.0) containing L-alanine (223 mg, 250 mM) and D-glucose monohydrate (297 mg, 150 mM), ammonium formate (94.5 mg, 150 mM), NADH disodium salt (7.1 mg, 1 mM final concentration), pyridoxal phosphate monohydrate (2.6 mg, 1 mM final concentration) and sterile empty vector *E. coli* BL21-(DE3) crude extract (30%, v/v) was added to the glass inlet.

Styrene (**1**, 57.4 µL, 500 µmol, 50 mM) was added, the reaction solution was stirred at 800 rpm (Teflon-coated stirring bar, 1 cm), and the reactor was pressurized for 1 min with 50 bar nitrogen (pressure holding test). In the next step, the autoclave was purged with nitrogen (3x with ~20 bar) and hydrogen (3x with ~20 bar). Subsequently, the autoclave was pressurized with 20 bar CO/$H_2$ (1:1) and heated to 30 °C. After 18 h, the autoclave was depressurized and purged with nitrogen (3x with 30 bar).

Please note: Pressurization and depressurization were performed slowly, as the organic compounds were volatile and dissolved in water to avoid stripping the compound.

The reaction mixture was extracted with ethyl acetate (10 mL) and centrifuged (1 min, 4000x g) for phase separation. The organic phase was dried over $CaCl_2$. Subsequently, the organic phase (100 µL) was diluted with 900 µL ethyl acetate and analyzed by GC. Removal of the solvent gave a colorless oil, which was analyzed using $^1$H-NMR spectroscopy.



**2**



**3**

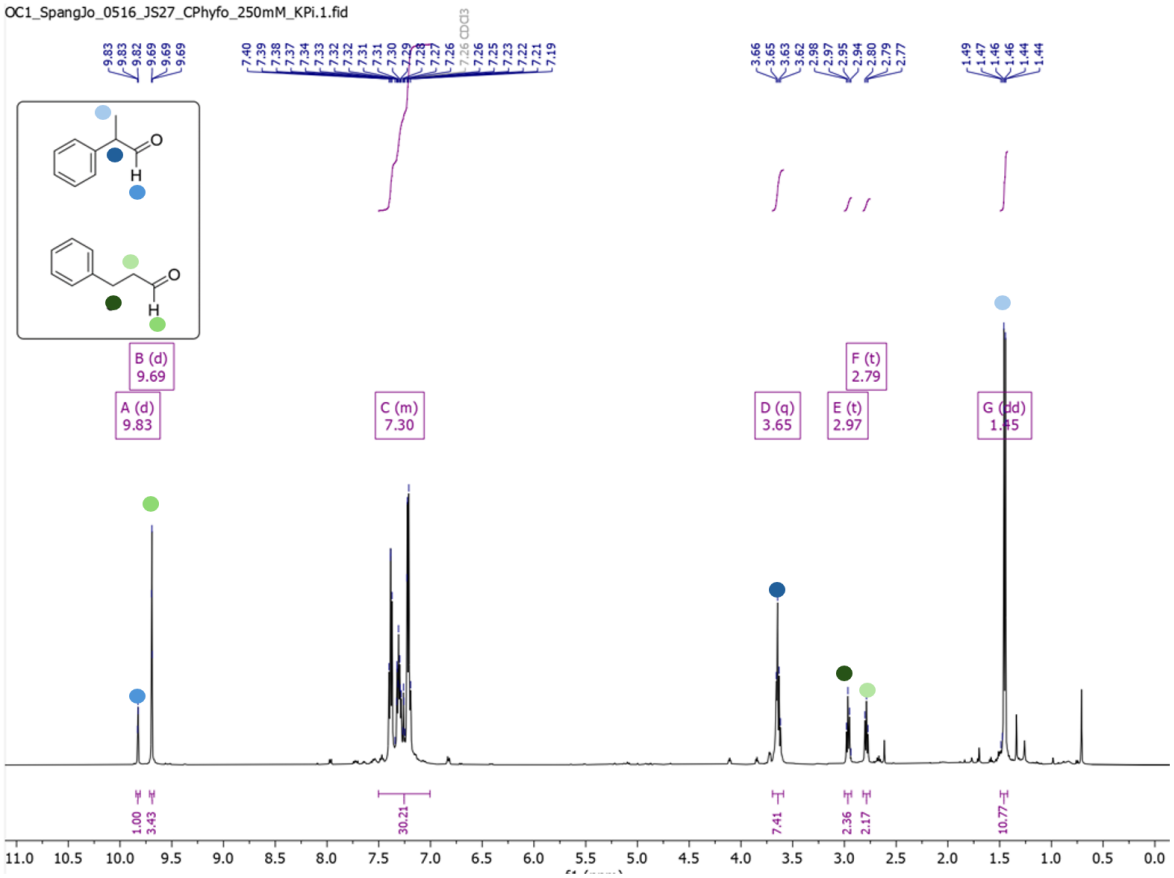
$^1$H-NMR (500 MHz, $CDCl_3$) $\delta$ = 9.86 (d, $J$ = 1.5 Hz, 1H, CHO); 9.73 (d, $J$ = 1.4 Hz, 1H, CHO); 7.50 – 7.13 (m, 10H, $H_{arom}$); 3.67 (q, $J$ = 6.7 Hz, 1H, CH); 3.00 (t, $J$ = 7.5 Hz, 2H, $CH_2$); 2.82 (t, $J$ = 7.6 Hz, 2H, $CH_2$); 1.49 (dd, $J$ = 7.0, 1.2 Hz, 3H, $CH_3$) ppm.

**Table 5:** Conversions and selectivities obtained from the hydroformylation of styrene (**1**) are presented. Hydroformylation of styrene (**1**) yielded *rac*-2-phenylpropanal (**2**) and 3-phenylpropanal (**3**).

| Substrate | Buffer and additives | Conversion / %[b] | Selectivity Product 2 / %[c] | Selectivity Product 3 / %[d] |
|---|---|---|---|---|
| **1** | PPB (250 mM, pH 7), additives[a] | 96 ± 3 | 76 ± 6 | 24 ± 6 |

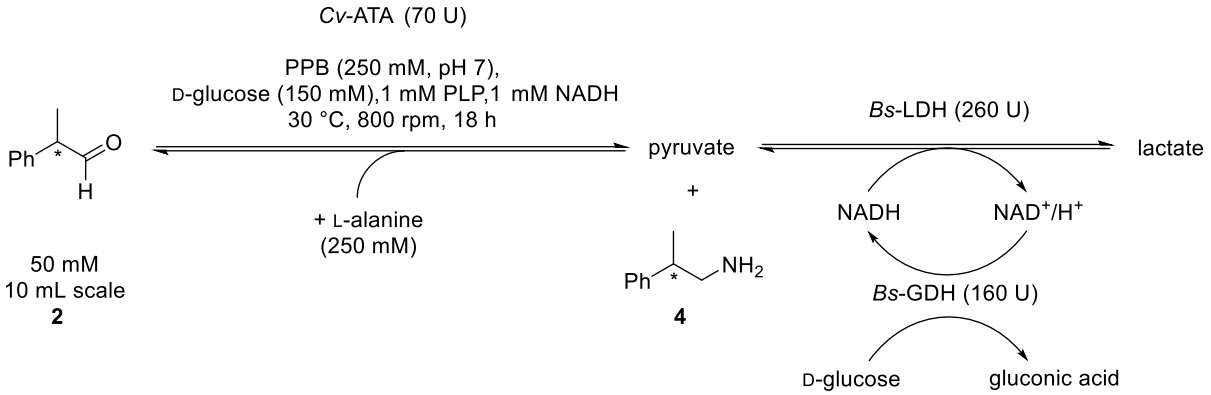
[a] Hydroformylation was conducted utilizing 0.2 wt% Triton X-100, 0.68 mol% [Rh(acac)$(CO)_2$], and 3.6 mol% 6-DPPon. Additionally, the additives 250 mM L-alanine, 150 mM D-glucose, 150 mM ammonium formate, 1 mM NADH, 1 mM PLP, 0.2 wt% Triton X-100, and empty vector *E. coli* BL21-(DE3) crude extract (30%, v/v) were included.
[b] Conversion was determined by GC in triplicate. Definition: $(n_{n\text{-product}} + n_{iso\text{-product}}) / (n_{product,\ theoretical})$.
[c] Selectivity was determined by GC in triplicate. Definition: $n_{iso\text{-product}} / (n_{iso\text{-product}} + n_{n\text{-product}})$.
[d] Selectivity was determined by GC in triplicate. Definition: $n_{n\text{-product}} / (n_{iso\text{-product}} + n_{n\text{-product}})$.
With n = amount of substance. The maximum error was estimated in all cases.

## 5.1 Reaction control *via* $^{1}$H-NMR



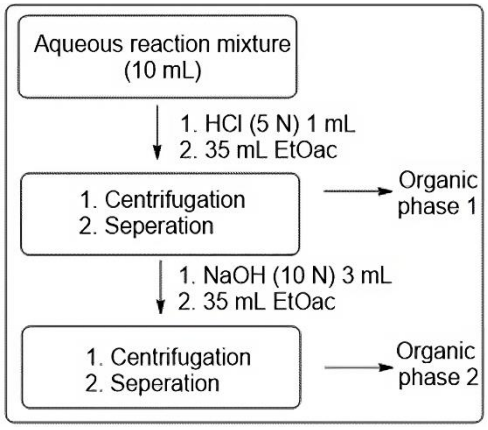

**S 8:** $^{1}$H-NMR spectrum in $CDCl_3$ (reaction control) of the hydroformylation crude product of styrene (**1**). The hydroformylation was conducted in potassium phosphate buffer (250 mM, pH 7) containing respective additives, utilizing the $Rh(acac(CO)_2$ precatalyst, 6-DPPon ligand, and Triton X-100 surfactant.

## 6 Biotransformation

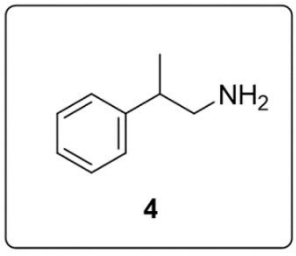



**S 9:** *Cv*-ATA catalyzed biotransformations of *rac*-2-phenylpropanal (**2**) to 2-phenylpropan-1-amine (**4**) at 50 mM substrate loading at 10 mL preparative scale. *Cv*-ATA catalyzed the biotransformation of aldehyde **2** to amine **4** combined with *Bs*-LDH-catalyzed *in situ* pyruvate removal. NADH recycling was facilitated by *Bs*-GDH.

**Procedure:** The biotransformation of the *rac*-2-phenylpropanal (**2**) was conducted in triplicate utilizing glass vials with a total reaction volume of 10 mL. Initially, potassium phosphate buffer (PPB, 250 mM, pH 7) containing L-alanine (223 mg, 250 mM final concentration), NADH disodium salt (7.1 mg, 1 mM final concentration), PLP monohydrate (2.6 mg, 1 mM final concentration), and D-glucose monohydrate (297 mg, 150 mM final concentration) was added to the glass vial. Subsequently, the crude extracts of *Cv*-ATA (70 U), *Bs*-LDH (260 U), and *Bs*-GDH (160 U) were added to the reaction solution. In the next step, the *rac*-2-phenylpropanal (**2**, 67 mg, 65 µL, 500 µmol) was added, and the reaction was carried out with stirring at 800 rpm (Teflon-coated stirring bar, 0.5 cm) at 30 °C for 18 h.




Subsequently, the reaction mixture was extracted according to the extraction scheme shown left. First, the reaction mixture was acidified (1 mL aqueous HCl, 5 N) and extracted with ethyl acetate (35 mL, OP1). After centrifugation for phase separation (1 min, 4000x g) and phase separation, the aqueous phase was basified (3 mL aqueous NaOH, 10 N) and extracted again with ethyl acetate (35 mL, OP2). Each organic phase (50 µL) was diluted with ethyl acetate (150 µL) and analyzed by GC. Removal of the solvent from the organic phase OP2 gave a yellowish oil, which was analyzed using $^1$H-NMR spectroscopy.



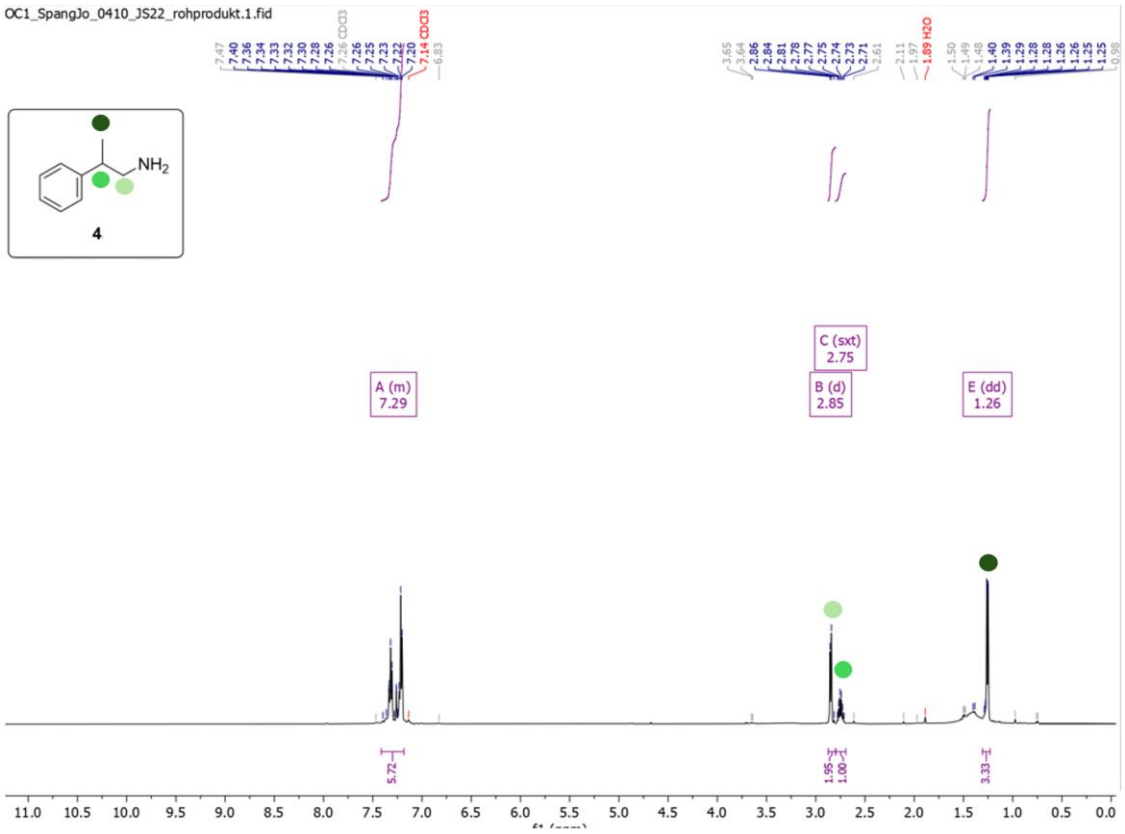

$^1$H-NMR (500 MHz, $CDCl_3$) $\delta$ = 7.41 – 7.18 (m, 5H, $H_{arom}$); 2.85 (d, $J$ = 7.0 Hz, 2H, $CH_2$); 2.75 (sxt, 1H, CH); 1.26 (dd, $J$ = 6.9, 1.8 Hz, 3H, $CH_3$) ppm.

**Table 6:** Conversion of *rac*-2-phenylpropanal (**2**) to 2-phenylpropan-1-amine (**4**) of the biotransformations at 50 mM substrate loading 10 mL preparative scale.

| Entry | Substrate | Product | Buffer and additives | TA | NADH recycling | LDH | Conv. / %[a] | *e.r.*[b] |
|---|---|---|---|---|---|---|---|---|
| **1** | **1** | **4** | PPB (250 mM, pH 7) with 1 mM NADH, 1 mM PLP, 250 mM L-alanine, 250 mM D-glucose | *Cv*-ATA (70 U) | *Cb*-GDH (160 U) | *Bs*-LDH (260 U) | 86 ± 9 | 52 : 48 (*R*) |

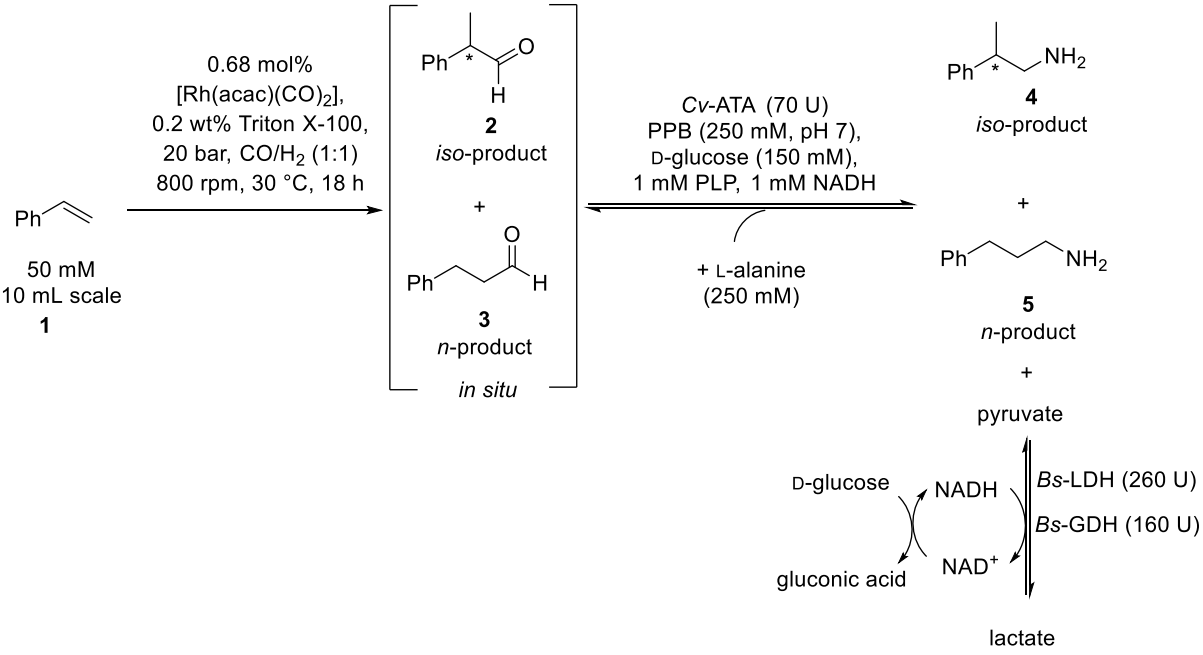
[a] Conversion was determined by GC in triplicate. Defined as ($n_{4a}$) / ($n_{product,\ theoretical}$); n = amount of substance. The reaction was conducted in triplicate, and the maximum error was estimated.
[b] Enantiomeric ratio (*e.r.*) was determined by chiral HPLC. Definition: (*(R)*/(*(S)*+*(R)* · 100%) : (*(S)*/(*(S)*+*(R)* · 100%).

## 6.1 Reaction control *via* $^{1}$H-NMR




**S 10:** $^{1}$H-NMR spectrum in $CDCl_3$ (reaction control) of the crude product obtained from the biotransformation of *rac*-2-phenylpropanal (**2**) to 2-phenylpropan-1-amine (**4**) in potassium phosphate buffer (pH 7, 250 mM) applying *Cv*-ATA, *Bs*-LDH, *Bs*-GDH at a 10 mL scale.

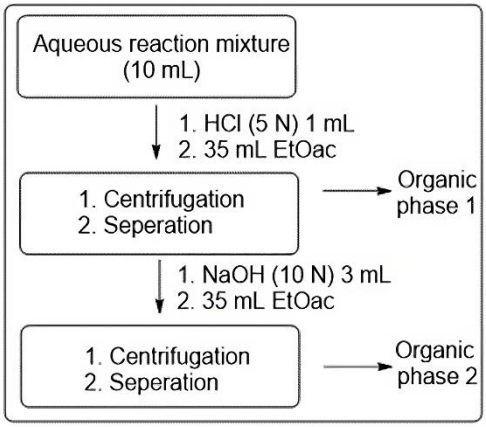
## 7 Chemoenzymatic primary amine synthesis



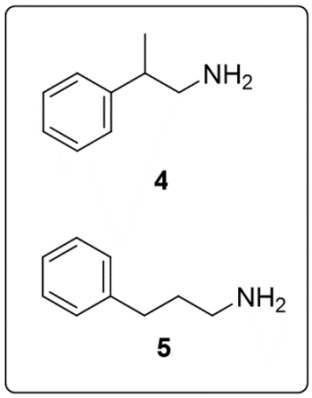

**S 11:** Chemoenzymatic synthesis of primary amines **4** and **5** in a one-pot process. The 6-DPPon/Rh-catalyzed hydroformylation of styrene (**1**) was combined with *Cv*-ATA-catalyzed transamination of *in situ* formed aldehydes. *Bs*-LDH was utilized for *in situ* pyruvate removal, and *Bs*-GDH was used for NADH recycling. The reaction was carried out in an aqueous biphasic system using the surfactant Triton X-100.

**Procedure:** The chemoenzymatic synthesis of primary amines was carried out in fivefold determination utilizing commercially available autoclaves (Series 5000 Multiple Reactor System with 75 mL vessels from Parr Instrument Company) with glass inlets at a total reaction volume of 10 mL under inert conditions.
In the first step, the [Rh(acac)$(CO)_2$] (0.9 mg, 3.4 µmol, 0.68 mol%), 6-DPPon (5.0 mg, 18 µmol, 3.6 mol%), and Triton X-100 (20 mg, 0.2 wt%) were weighed directly in the glass inlet. The autoclave was sealed, vacuumed, and purged with argon (3x) with the glass inlet inserted. Under an argon atmosphere, degassed potassium phosphate buffer (PPB, 250 mM, pH 7.0) containing L-alanine (223 mg, final concentration 250 mM), pyridoxal phosphate monohydrate (2.6 mg, final concentration 1 mM), D-glucose monohydrate (297 mg, 150 mM final concentration), and NADH disodium salt (7.1 mg, final concentration 1 mM) was added. Subsequently, the crude extracts of the *Cv*-ATA (70 U), the LDH (260 U), and the *Bs*-GDH (160 U) were added to the reaction solution. Styrene (**1,** 57.4 µL, 500 µmol, 50 mM) was added, the reaction solution was stirred at 800 rpm (Teflon-coated stirring bar, 1 cm), and the reactor was pressurized for 1 min with ~30 bar nitrogen (pressure holding test). In the next step, the autoclave was purged with nitrogen (3x with ~20 bar) and hydrogen (3x with ~20 bar). Subsequently, the autoclave was pressurized with 20 bar $CO/H_2$ (1:1) and heated to 30 °C for 18 h. After the reaction time, the autoclave was depressurized and purged with nitrogen (3x with 30 bar).

Please note: Pressurization and depressurization were performed slowly, as the organic compounds were volatile and dissolved in water to avoid stripping the compound.




Subsequently, the reaction mixture was extracted according to the extraction scheme shown left. First, the reaction mixture was acidified (1 mL aqueous HCl, 5 N) and extracted with ethyl acetate (35 mL, OP1). After centrifugation for phase separation (1 min, 4000x g), the aqueous phase was basified (3 mL aqueous NaOH, 10 N) and extracted again with ethyl acetate (35 mL, OP2). Each organic phase (50 µL) was diluted with ethyl acetate (150 µL) and analyzed by GC. The solvent of the organic phase OP2 was evaporated under reduced pressure and the crude product was received as a yellowish oil. The crude product was dissolved in ethyl acetate containing 5% triethylamine and purified *via* preparative thin-layer chromatography (TLC). The purified compounds were subjected to $^{1}$H-NMR spectroscopy. Furthermore, the purified compounds were dissolved in cyclohexane (1 mg/mL), and the enantiomeric ratio was determined *via* chiral HPLC.




Chemoenzymatic synthesis yielded 51.7% of the *iso*-product **4** (34.7 mg).

$^{1}$H-NMR (500 MHz, $CDCl_3$) $\delta$ = 7.41 – 7.13 (m, 5H, $H_{arom}$); 2.85 (s, 2H, $CH_2$); 2.75 (p, $J$ = 6.9 Hz, 1H, CH); 1.26 (d, $J$ = 6.9 Hz, 3H $CH_3$) ppm.

$R_f$ = 0.35

Chemoenzymatic synthesis yielded 8.0% of the *n*-product **5** (5.4 mg).

$^{1}$H-NMR (500 MHz, $CDCl_3$) $\delta$ = 7.34 – 7.16 (m, 5H, $H_{arom}$); 2.77 (t, $J$ = 7.1 Hz, 2H, $CH_2$); 2.67 (t, $J$ = 7.7 Hz, 2H, $CH_2$); 1.83 (p, $J$ = 7.4 Hz, 2H, $CH_2$) ppm.

$R_f$ = 0.23

Combined, the chemoenzymatic synthesis yielded 60% of amines **4** and **5** (40.1 mg).

**Table 7:** Conversions and selectivities obtained from the chemoenzymatic primary amine synthesis starting from styrene (**1**). The chemoenzymatic synthesis yielded 2-phenylpropan-1-amine (**4**) and 3-phenylpropan-1-amine (**5**).

| **Substrate** | **Buffer and additives** | **TA** | **NADH recycling** | **LDH** | **Conv. / %**[b] | **Select. 4 / %**[c] | **Select. 5 / %**[d] | ***e.r.***[e] / % |
|---|---|---|---|---|---|---|---|---|
| **1** | PPB (250 mM, pH 7) with additives[a] | *Cv*-ATA (70 U) | *Bs*-GDH (160 U) | *Bs*-LDH (260 U) | >99 ± 6 | 79 ± 6 | 21 ± 6 | 56 : 44 (*R*) |

[a] Additives: 1 mM NADH, 1 mM PLP, 250 mM L-alanine, 0.2 wt% Triton X-100, 0.68 mol% $[Rh(acac)(CO)_2]$, 3.6 mol% 6-DPPon.

[b] Conversion was determined by GC in fivefold determination. Definition: $(n_4 + n_5) / (n_{product,\ theoretical})$.

[c] Selectivity was determined by GC in fivefold determination. Definition: $n_4 / (n_4 + n_5)$.

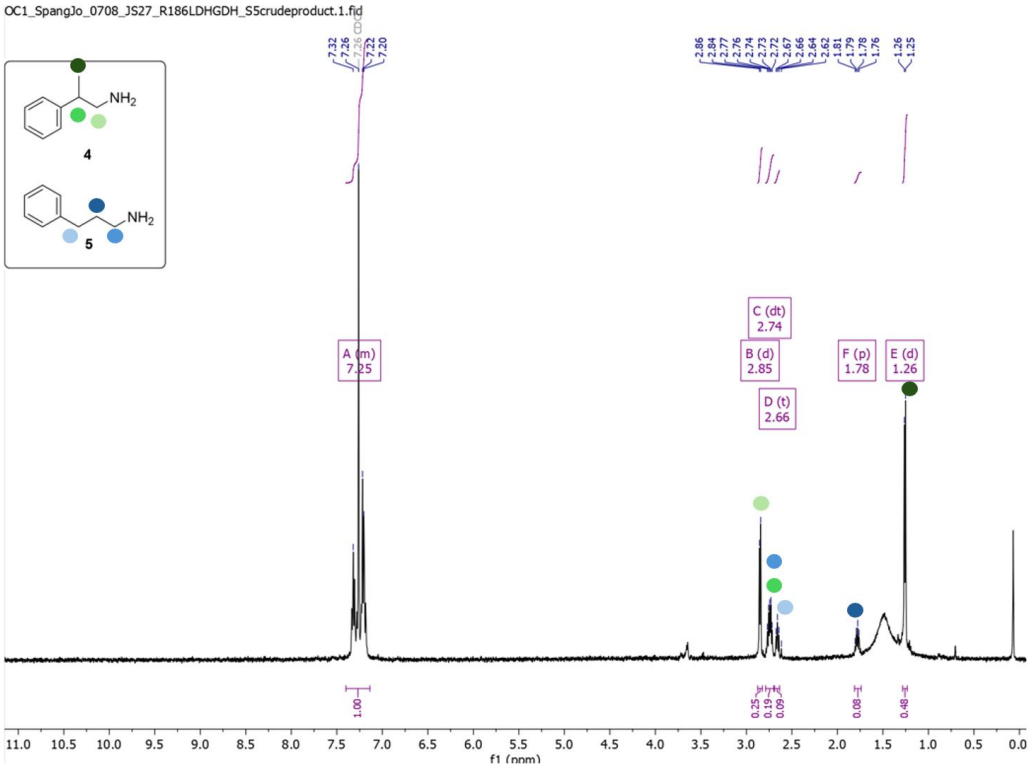
[d] Selectivity was determined by GC in fivefold determination. Definition: $n_5 / (n_4 + n_5)$.

With n = amount of substance. The maximum error was estimated in all cases.

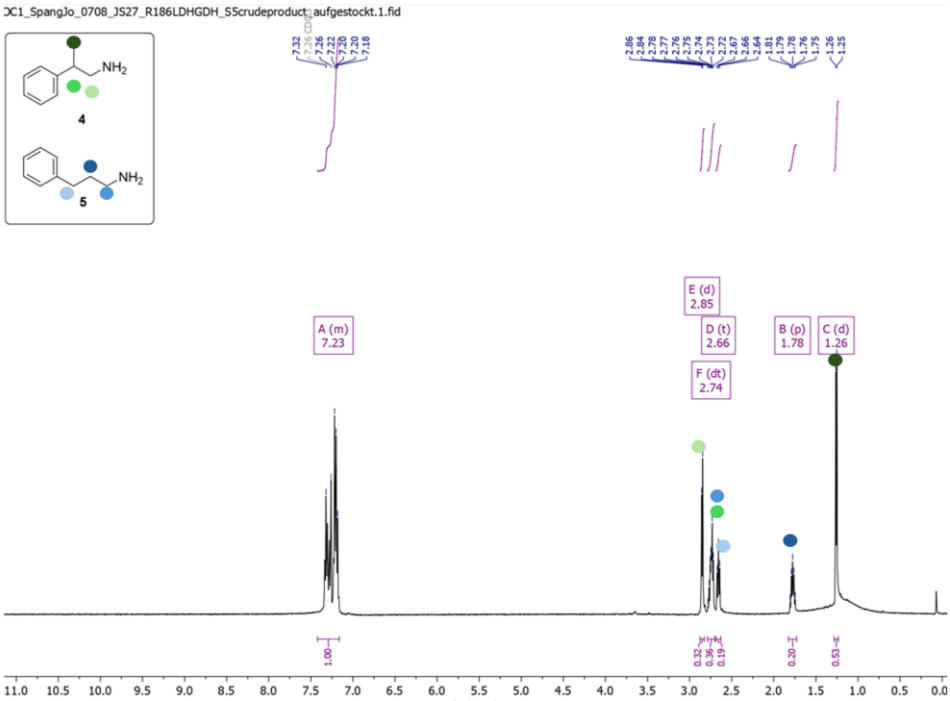
[e] Enantiomeric ratio (*e.r.*) was determined by chiral HPLC. Definition:

$(R)/((S)+(R) \cdot 100\%) : (S)/((S)+(R) \cdot 100\%)$.

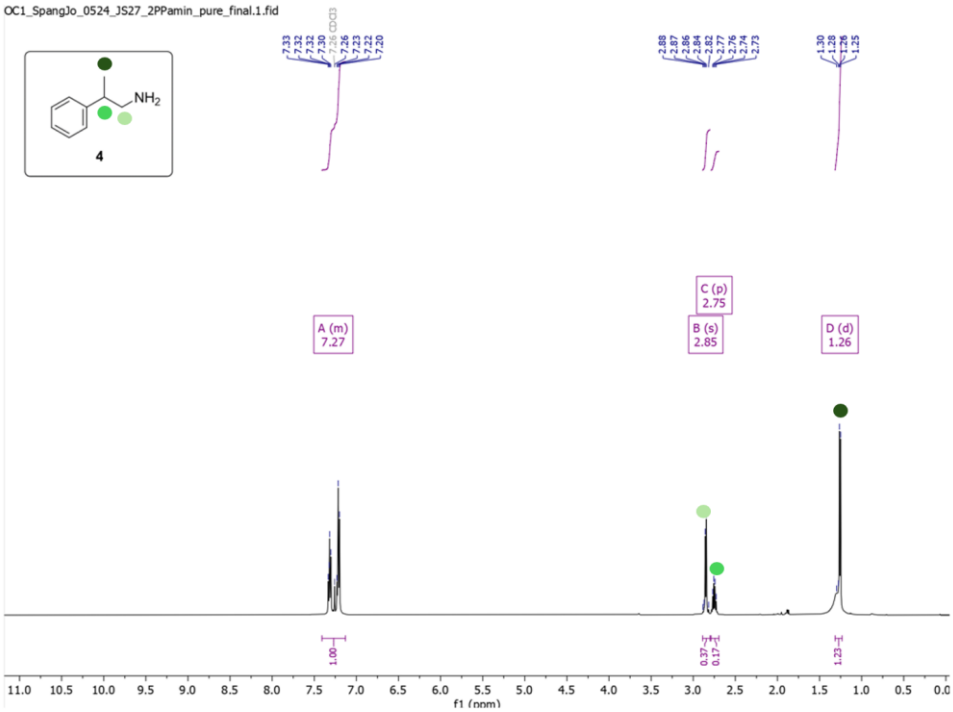
## 7.1 Reaction control *via* $^{1}$H-NMR



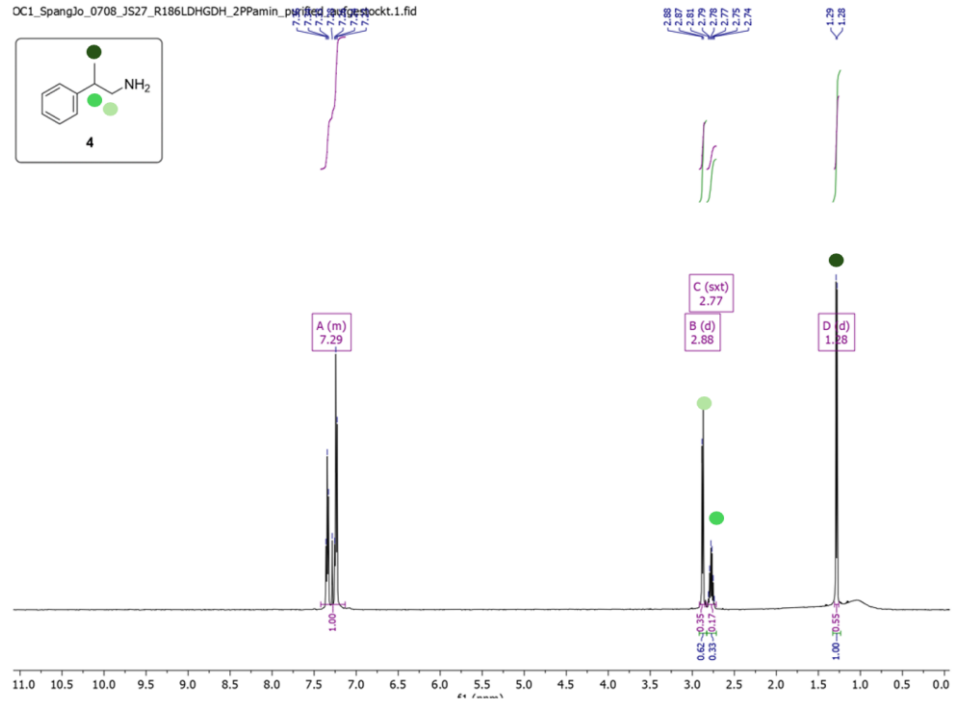

**S 12:** $^{1}$H-NMR spectrum in $CDCl_3$ of the **crude product** obtained from chemoenzymatic synthesis starting from styrene (**1**). The hydroformylation step was conducted utilizing the [Rh(acac)$(CO)_2$] precatalyst, 6-DPPon ligand, and Triton X-100 surfactant. The biotransformation step was carried out using *Cv*-ATA, *Bs*-LDH and *Bs*-GDH.



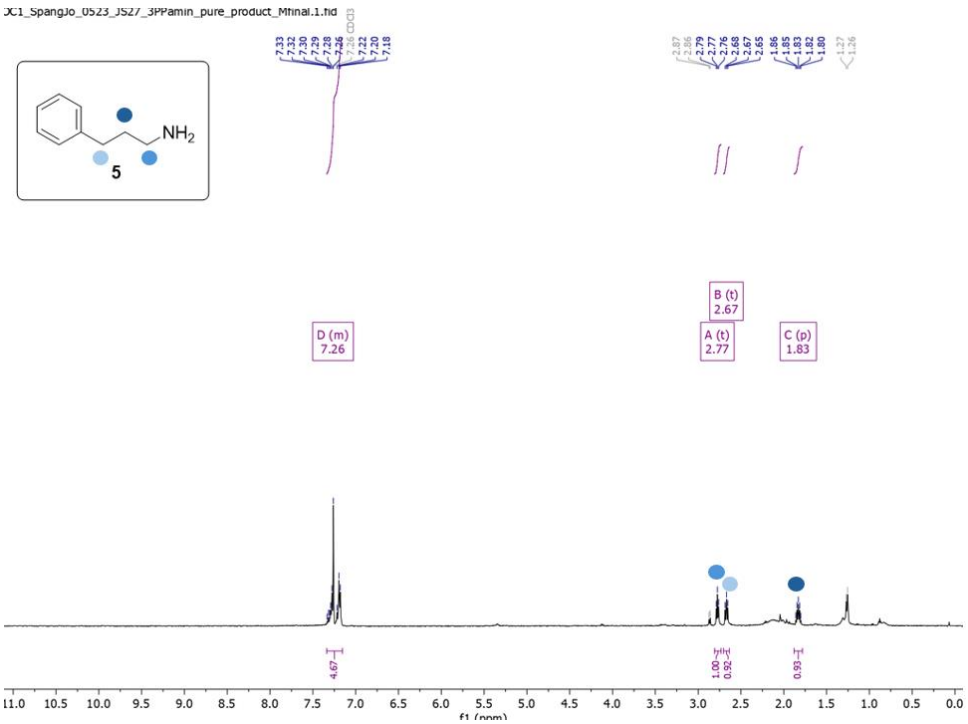

**S 13:** $^{1}$H-NMR spectrum in $CDCl_3$ of the **crude product** obtained from chemoenzymatic synthesis starting from styrene (**1**). The hydroformylation step was conducted utilizing the [Rh(acac)$(CO)_2$] precatalyst, 6-DPPon ligand, and Triton X-100 surfactant. The biotransformation step was carried out using *Cv*-ATA, *Bs*-LDH and *Bs*-GDH. The $^{1}$H-NMR spectrum was **spiked** with the 2-phenylpropan-1-amine (**4**) and 3-phenylpropan-1-amine (**5**) references.



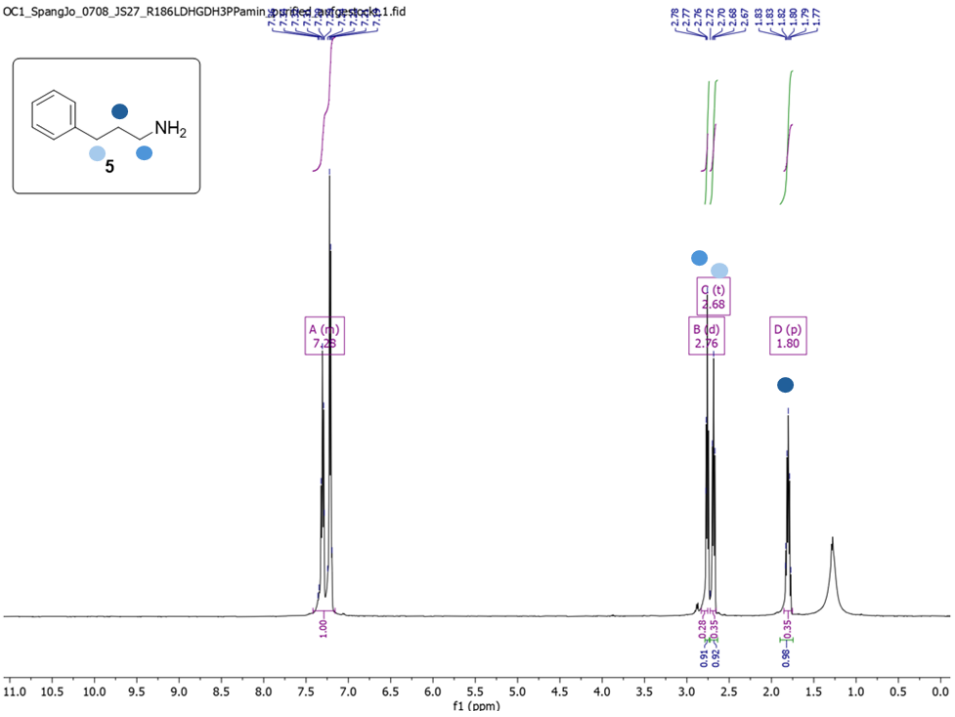

**S 14:** $^{1}$H-NMR spectrum in $CDCl_3$ of the **purified** 2-phenylpropan-1-amine (**4**) obtained from chemoenzymatic synthesis starting from styrene (**1**). The hydroformylation step was conducted utilizing the [Rh(acac)(CO)$_2$] precatalyst, 6-DPPon ligand, and Triton X-100 surfactant. The biotransformation step was carried out using *Cv*-ATA, *Bs*-LDH and *Bs*-GDH.




**S 15:** $^{1}$H-NMR spectrum of the **purified** 2-phenylpropan-1-amine (**4**) obtained from chemoenzymatic synthesis starting from styrene (**1**). The hydroformylation step was conducted utilizing the [Rh(acac)(CO)$_2$] precatalyst, 6-DPPon ligand, and Triton X-100 surfactant. The biotransformation step was carried out using *Cv*-ATA, *Bs*-LDH and *Bs*-GDH. The $^{1}$H-NMR spectrum was **spiked** with the 2-phenylpropan-1-amine (**4**) reference.



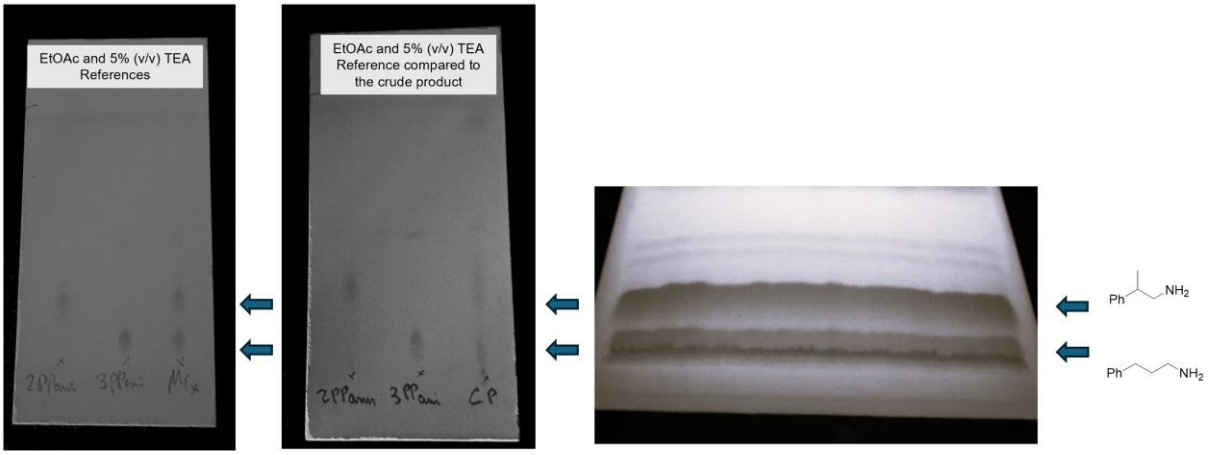

**S 16:** $^{1}$H-NMR spectrum in $CDCl_3$ of the **purified** 3-phenylpropan-1-amine (**5**) obtained from chemoenzymatic synthesis starting from styrene (**1**). The hydroformylation step was conducted utilizing the [Rh(acac)$(CO)_2$] precatalyst, 6-DPPon ligand, and Triton X-100 surfactant. The biotransformation step was carried out using *Cv*-ATA, *Bs*-LDH and *Bs*-GDH.




**S 17:** $^{1}$H-NMR spectrum of the **purified** 3-phenylpropan-1-amine (**5**) obtained from chemoenzymatic synthesis starting from styrene (**1**). The hydroformylation step was conducted utilizing the [Rh(acac)$(CO)_2$] precatalyst, 6-DPPon ligand, and Triton X-100 surfactant. The biotransformation step was carried out using *Cv*-ATA, *Bs*-LDH and *Bs*-GDH. The $^{1}$H-NMR spectrum was **spiked** with the 3-phenylpropan-1-amine (**5**) reference.

## 8 Thin-layer chromatography

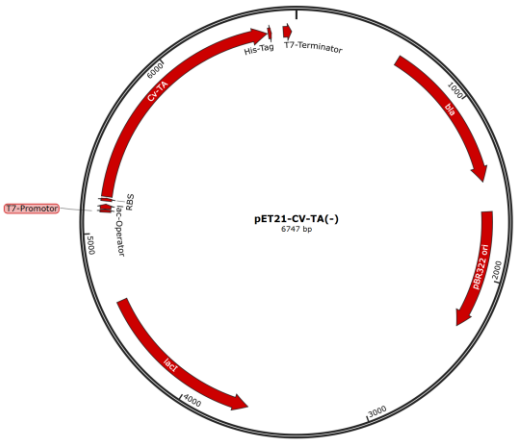
**Thin-layer chromatography:** The thin-layer chromatography was performed on Alugram SIL G/$UV_{254}$ plates from Macherey-Nagel (0.20 mm silica gel 60 with fluorescent indicator, 40 mm x 80 mm) using ethyl acetate containing 5% (v/v) triethylamine as a solvent and a UV-lamp to visualize the compounds.
**Preparative thin-layer chromatography:** The preparative thin-layer chromatography was performed on SIL G-100 $UV_{254}$ plates from Macherey-Nagel (1 mm silica gel 60 with fluorescent indicator, 20 cm x 20 cm) using ethyl acetate containing 5% (v/v) triethylamine as a solvent and a UV-lamp to visualize the compounds.




**S 18:** Representative chromatograms of thin-layer chromatography of the crude product obtained from chemoenzymatic primary amine synthesis starting from styrene (*cf.* 5.6.2). $R_f$ = 0.35 2-phenylpropan-1-amine; $R_f$ = 0.23 of 3-phenylpropan-1-amine. CP = crude product obtained from chemoenzymatic synthesis. 2-PPamin = 2-Phenylpropan-1-amine. 3-PPamin = 3-phenylpropan-1-amine.

# 9 Plasmids and sequences

***Chromobacterium violaceum Bergonzini* 1880 DSMZ 30191 aminotransaminase (Cv-ATA)**
Internal protein number: R186
MW: 51.2 kDa




**S 19:** Illustration of pET21a(-) plasmid with *Cv*-ATA gene.

Amino acid sequence:

MQKQRTTSQWRELDAAHHLHPFTDTASLNQAGARVMTRGEGVYLWDSEGNKIIDGMAGLWCVNVGYGRKDFAEAARRQMEEL
PFYNTFFKTTHPAVVELSSLLAEVTPAGFDRVFYTNSGSESVDTMIRMVRRYWDVQGKPEKKTLIGRWNGYHGSTIGGASLGGMK
YMHEQGDLPIPGMAHIEQPWWYKHGKDMTPDEFGVVAARWLEEKILEIGADKVAAFVGEPIQGAGGVIVPPATYWPEIERICRKY
DVLLVADEVICGFGRTGEWFGHQHFGFQPDLFTAAKGLSSGYLPIGAVFVGKRVAEGLIAGGDFNHGFTYSGHPVCAAVAHANVA
ALRDEGIVQRVKDDIGPYMQKRWRETFSRFEHVDDVRGVGMVQAFTLVKNKAKRELFPDFGEIGTLCRDIFFRNNLIMRACGDHI
VSAPPLVMTRAEVDEMLAVAERCLEEFEQTLKARGLA*

DNA sequence:

ATGCAGAAACAGCGTACCACCTCTCAGTGGCGTGAACTGGATGCAGCACATCATCTGCATCCGTTTACCGATACCGCAAGCCT
GAATCAGGCAGGTGCACGTGTTATGACCCGTGGTGAAGGTGTTTATCTGTGGGATAGCGAAGGCAACAAAATTATTGATGGT
ATGGCAGGTCTGTGGTGTGTTAATGTTGGTTATGGTCGCAAAGATTTTGCAGAAGCAGCACGTCGTCAGATGGAAGAACTGCC
GTTTTATAATACCTTTTTTAAAACCACCCATCCGGCAGTTGTTGAACTGAGCAGCCTGCTGGCCGAAGTTACACCGGCAGGTT
TTGATCGTGTGTTTTATACCAATAGCGGTAGCGAAAGCGTTGATACCATGATTCGCATGGTTCGTCGTTATTGGGATGTTCAG
GGCAAACCGGAAAAAAAAAACCCTGATCGGTCGTTGGAATGGTTATCATGGTAGCACCATTGGTGGTGCCAGCCTGGGTGGTA
TGAAATATATGCATGAACAGGGTGATCTGCCGATTCCGGGTATGGCACATATTGAACAGCCGTGGTGGTATAAACATGGCAA
AGATATGACACCGGATGAATTTGGTGTTGTTGCAGCACGTTGGCTGGAAGAAAAAATTCTGGAAATTGGTGCCGATAAAGTT
GCAGCATTTGTGGGTGAACCGATTCAGGGTGCAGGTGGTGTTATTGTTCCGCCTGCAACCTATTGGCCTGAAATTGAACGTAT
CTGCCGCAAATATGATGTTCTGCTGGTTGCCGATGAAGTTATTTGTGGTTTTGGTCGTACAGGTGAATGGTTTGGTCATCAGCA
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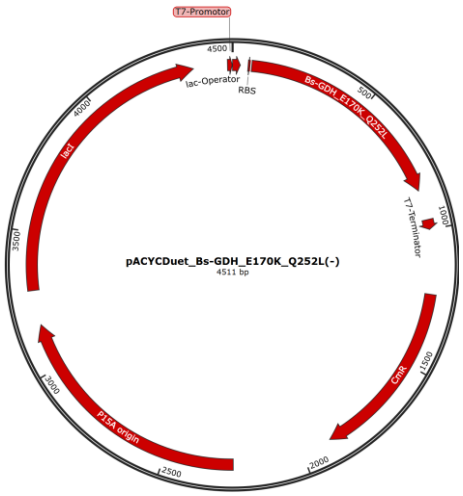
ACGTGTTGCAGAAGGTCTGATTGCAGGCGGTGATTTTAATCATGGCTTTACCTATAGCGGTCATCCGGTTTGTGCAGCAGTTG
CACATGCAAATGTTGCAGCACTGCGTGATGAAGGTATTGTTCAGCGCGTGAAAGATGATATTGGTCCGTATATGCAGAAACG
TTGGCGTGAAACCTTTAGCCGTTTTGAACATGTTGATGATGTTCGTGGTGTTGGTATGGTTCAGGCATTTACCCTGGTGAAAAA
CAAAGCAAAACGCGAACTGTTTCCGGATTTTGGTGAAATTGGCACCCTGTGCCGTGATATTTTTTTTCGCAATAATCTGATTAT
GCGTGCCTGTGGTGATCACATTGTTAGCGCACCGCCTCTGGTGATGACCCGTGCCGAAGTTGATGAAATGCTGGCCGTTGCAG
AACGCTGTCTGGAAGAATTTGAACAGACCCTGAAAGCACGTGGTCTGGCCTAA

***Bacillus subtilis 168 (ATCC 23857)* Glucose dehydrogenase_E170K_Q252L (*Bs*-GDH)**
Internal protein number: R65
MW: 28.1 kDa




**S 20:** Illustration of pACYCDuet plasmid with *Bs*-GDH gene (mutant_E170K_Q252L).

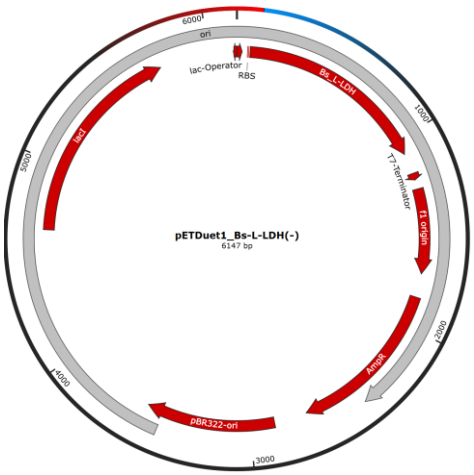
## Amino acid sequence:

MYPDLKGKVVAITGAASGLGKAMAIRFGKEQAKVVINYYSNKQDPNEVKEEVIKAGGEAVVVQGDVTKEEDVKNIVQTAIKEFG
TLDIMINNAGLENPVPSHEMPLKDWDKVIGTNLTGAFLGSREAIKYFVENDIKGNVINMSSVHEVIPWPLFVHYAASKGGIKLMTK
TLALEYAPKGIRVNNIGPGAINTPINAEKFADPKQKADVESMIPMGYIGEPEEIAAVAAWLASKEASYVTGITLFADGGMTLYPSFQ
AGRG*

## DNA sequence:

ATGTATCCGGATTTAAAAGGAAAAGTCGTCGCTATTACAGGAGCTGCTTCAGGGCTCGGAAAGGCGATGGCCATTCGCTTCGGC
AAGGAGCAGGCAAAAGTGGTTATCAACTATTATAGTAATAAACAAGATCCGAACGAGGTAAAAGAAGAGGTCATCAAGGCGGG
CGGTGAAGCTGTTGTCGTCCAAGGAGATGTCACGAAAGAGGAAGATGTAAAAAATATCGTGCAAACGGCAATTAAGGAGTTCG
GCACACTCGATATTATGATTAATAATGCCGGTCTTGAAAATCCTGTGCCATCTCACGAAATGCCGCTCAAGGATTGGGATAAAGT
CATCGGCACGAACTTAACGGGTGCCTTTTTAGGAAGCCGTGAAGCGATTAAATATTTCGTAGAAAACGATATCAAGGGAAATGT
CATTAACATGTCCAGTGTGCACGAAGTGATTCCTTGGCCGTTATTTGTCCACTATGCGGCAAGTAAAGGCGGGATAAAGCTGAT
GACAAAGACATTAGCGTTGGAATACGCGCCGAAGGGCATTCGCGTCAATAATATTGGGCCAGGTGCGATCAACACGCCAATCAA
TGCTGAAAAATTCGCTGACCCTAAACAGAAAGCTGATGTAGAAAGCATGATTCCAATGGGATATATCGGCGAACCGGAGGAGAT
CGCCGCAGTAGCAGCCTGGCTTGCTTCGAAGGAAGCCAGCTACGTCACAGGCATCACGTTATTCGCGGACGGCGGTATGACAC
TCTATCCTTCATTCCAGGCAGGCCGCGGTTAA

***Bacillus subtilis* 168 (ATCC 23857) lactate dehydrogenase (*Bs*-LDH)**
Internal protein number: R68
MW: 34.8 kDa




**S 21:** Illustration of pACYCDuet plasmid with *Bs*-LDH gene.

## Amino acid sequence:

MNKHVNKVALIGAGFVGSSYAFALINQGITDELVVIDVNKEKAMGDVMDLNHGKAFAPQPVKTSYGTYEDCKDADIVCICAGAN
QKPGETRLELVEKNLKIFKGIVSEVMASGFDGIFLVATNPVDILTYATWKFSGLPKERVIGSGTTLDSARFRFMLSEYFGAAPQNVH
AHIIGEHGDTELPVWSHANVGGVPVSELVEKNDAYKQEELDQIVDDVKNAAYHIIEKKGATYYGVAMSLARITKAILHNENSILTV
STYLDGQYGADDVYIGVPAVVNRGGIAGITELNLNEKEKEQFLHSAGVLKNILKPHFAEQKVN

## DNA sequence:

ATGAACAAACATGTAAATAAAGTAGCTTTAATCGGAGCGGGTTTTGTTGGAAGCAGTTATGCATTTGCGTTAATTAACCAAGGA
ATCACAGATGAGCTTGTGGTCATTGATGTAAATAAAGAAAAAGCAATGGGCGATGTGATGGATTTAAACCACGGAAAGGCGTTT
GCGCCACAACCGGTCAAAACATCTTACGGAACATATGAAGACTGCAAGGATGCTGATATTGTCTGCATTTGCGCCGGAGCAAAC
CAAAAACCTGGTGAGACACGCCTTGAATTAGTAGAAAAGAACTTGAAGATTTTCAAAGGCATCGTTAGTGAAGTCATGGCGAG
CGGATTTGACGGCATTTTCTTAGTCGCGACAAATCCGGTTGATATCCTGACTTACGCAACATGGAAATTCAGCGGCCTGCCAAA
AGAGCGGGTGATTGGAAGCGGCACAACACTTGATTCTGCGAGATTCCGTTTCATGCTGAGCGAATACTTTGGCGCAGCGCCTCA
AAACGTACACGCGCATATTATCGGAGAGCACGGCGACACAGAGCTTCCTGTTTGGAGCCACGCGAATGTCGGCGGTGTGCCGG
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AACAGCATATTAACTGTCAGCACATATTTGGACGGGCAATACGGTGCAGATGACGTGTACATCGGTGTGCCGGCTGTCGTGAAT
CGCGGAGGGATCGCAGGTATCACTGAGCTGAACTTAAATGAGAAAGAAAAAGAACAGTTCCTTCACAGCGCCGGCGTCCTTAA
AAACATTTTAAAACCTCATTTTGCAGAACAAAAAGTCAACT