Supporting Information Communication

Biocompatible metal-assisted C-C cross-coupling combined with biocatalytic chiral reductions in a concurrent tandem cascade

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1 General considerations

Unless noted otherwise, all reagents were purchased from commercial suppliers and used without further purification. Chromatography solvents were distilled prior to use. ^{1}H-NMR and ^{13}C-NMR were recorded from $CHCl_3$-d1 on a Bruker AC 200 (200 MHz), Bruker Advance UltraShield 400 (400 MHz) or Bruker Advance UltraShield 600 (600 MHz) spectrometer and chemical shifts are reported in ppm. UPC^2/ General conversion control and examination of purified products were conducted with Thermo Finnigan Focus GC / DSQ II using a standard capillary column BGB5 (30m x 0.32 mm ID) or a UPC^2/UV from Waters (Torus 1-AA 1,7 µm, 2.1 mm x 150 mm, A: scrCO_2, B: ACN, A: 99->75%, 2min, 0.7 mL flow rate, 1900 psi ABPR pressure). Enantiomeric excess was determined via GC using a BGB173 (30 m x 0.25 mm ID, 0.25 µm film) column on a ThermoQuest Trace GC 2000 and a ThermoFocus GC with FID detector. The injected sample volume was 1 µL. The alcohol dehydrogenases ADH-A from *Rhodococcus ruber* and LK-ADH from *Lactobacillus kefir* were used as whole cell lyophilisate. As buffer a Tris HCl buffer (320 mM) with pH 8.0 was applied for the biocatalytic reduction (in separate enzymatic reactions and in the respective reaction chamber in chemoenzymatic reactions). Specific rotation was measured on an Anton Paar MCP500 polarimeter at the specified conditions. GC-methods are depicted in Table S1. Synthesis and characterisation of reference ketones and racemic alcohol compounds are described elsewhere.[1]

Table S1 GC methods for chiral and achiral columns.

Method A	80°C-2min→ 80-160°C, 5°C/min, 160°C-1min→ 160-220°C, 10°C/min, 220°C-8min
Method B	80°C-1min→80-220°C, 2°C/min, 220°C-5min
Method C	80°C-1min→ 80-220°C, 1°C/min, 220°C-5min
Method D	80°C-1min→ 80-90°C, 5°C/min, 90°C-12min→ 90-220°C, 10°C/min, 220°C-5min
Method E	65°C-0.5min→ 65-220°C, 25°C/min, 220°C-0min→ 220-290°C, 80°C/min, 290°C-3min

2 Expression of alcohol dehydrogenases

2.1 Preparation of lyophilized cells of E. coliBL21(DE3)/pET22b(+)_adh-A (from Rhodococcus ruber)

E. coli BL21(DE3)/pET22b(+)_*adh-A*was stored at -80°C in LB-amp containing 25% (v/v) glycerol. Prior to use, cells were plated on LB-amp plates (100 µg·mL^{-1} final ampicillin concentration). A single colony was used to inoculate 200 mL of TB-amp (100 µg·mL^{-1} final ampicillin concentration) in a 1 L baffled shake flask.$ZnCl_2$ was added from a 100 mM stock to a final concentration of 1 mM and cells were grown at 30°C with shaking (120 rpm) for about 20 h. On the next day OD_{590} was checked ($OD5_{90}$~6.0) and 200 µL of ampicillin (50 mg·mL^{-1} stock) were added. Protein production was induced upon addition of IPTG from a 100 mM stock to a final concentration of 2 mM and cells were cultivated at 20°C with shaking (120 rpm) for 24 h. Cells were harvested by centrifugation (6 000 x g, 15 min, 4°C). The medium was discarded; cells were re-suspended in sterile water, snap frozen in liquid nitrogen and lyophilized**.**

2.2 Preparation of lyophilized cells of *E. coli*BL21(DE3)/pET21b(+)_*LK-ADH* (from Lactobacillus *kefir*)

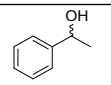
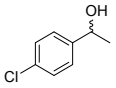
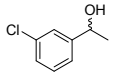
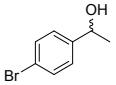
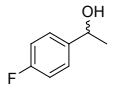
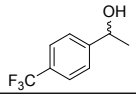
E. coli BL21(DE3)/pET21b(+)_*LK-ADH* was stored at -80°C in LB-amp containing 25% (v/v) glycerol. Prior to use, cells were plated on LB-amp plates (100 µg·mL^{-1} final ampicillin concentration). A single colony was used to prepare 4 mL of an overnight culture in LB medium containing 100 µg·mL^{-1} ampicillin (added from a 50 mg·mL^{-1} stock). The main culture was prepared by inoculation of 200 mL TB-amp (100 µg·mL^{-1} final ampicillin concentration) in a 1 L baffled shake flask with 2 mL of the overnight culture. Cells were grown at 37°C with shaking (120 rpm) to an OD_{590} = 0.5. Enzyme expression was induced by the addition of IPTG (1 mM final concentration) from a 100 mM IPTG stock. Cells were cultivated at 30°C with shaking (120 rpm) and harvested by centrifugation (6 000 x g, 15 min, 4°C) after 24 h. The medium was discarded; cells were re-suspended in sterile water, snap frozen in liquid nitrogen and lyophilized**.**

3 General protocol for biocatalytic reduction

Lyophilized cells of *E. coli* ADH-A (10 mg) respective LK-ADH (20 mg) were rehydrated in Tris HCl buffer (pH 8, 320mM, 412 µl) for 1 h at 30°C with 200 rpm in Eppendorf tubes (1.5 ml). Substrate (100mM, 5.8 µl) and 2-propanol (83 µl) were added and the mixture was agitated at 30°C and 200 rpm for 24 hours. The reaction was stopped by addition of ethyl acetate (10 µl reaction mixture extracted in 0.2 ml ethyl acetate). The organic layer

was separated from the aqueous phase by centrifugation and dried over Na_2SO_4. Conversions and enantioselectivities were determined by GC analysis (Table S2).

Table S2 Substrate screening applying enantiodivergent alcohol dehydrogenases.

Product		ADH-A Conversion [%] 4h	ee [%]	LK-ADH Conversion [%] 4 h	ee [%]
	**4a**	95	99	80	99
	**4b**	98	99	89	99
	**4c**	97	99	88	99
	**4d**	92	99	71	99
	**4e**	99	99	83	99
	**4e**	99	99	99	99

4 Biocompatibility studies of ω–TA ATA025 and L.-S. coupling components

After we had successfully applied the compartmentalization concept for the combination of L.-S. coupling and two enantiodivergent ADHs we focused on the the biocatalytic reduction via a ω–TA. Unfortunately, no conversion was observed. Hence, we performed an intensive parameter screening and elucidated the critical coupling components, which inhibited the biocatalytic reaction. The reaction conditions were set to 10mM Ketone, 37°C, 40 mg ATA025, 1M isopropylamine. The results are summarized in Table S3.

Table S3 Biocompatibility study of ω–TA in the presence of L.-S. coupling components.

Entry	**Addition of**	**Conversion (GC)[a] 24 h [%]**
1	*S*-phenyl thioacetate [100 mM]	0
2	*S*-*tert*-butyl thioacetate [100 mM]	62
3	Phenylboronic acid [170 mM]	0
4	$Pd_2(dba)_3$ [2.5 mol %]	78
5	Triethylphosphite [20 mol %]	8
6	Triethylphosphite [10 mol %]	18
7	Thiophene-2-carboxylic acid [160 mM]	47
8	Heptane [60 %]	59

[a]Quantification after pH-value adjustment to ≥11, extraction of the overall biphasic reaction with EtOAc and subsequent GC-analysis.

5 Preparation of the PDMS sheets

PDMS sheets were prepared according to Bowden with some modifications.[2]

5.1 Precuring

The elastomer and curing agent (Sylgard 184, 10 gram clip-pak, purchased from Sigma-Aldrich) were mixed and stand for 2 hours for degassing.

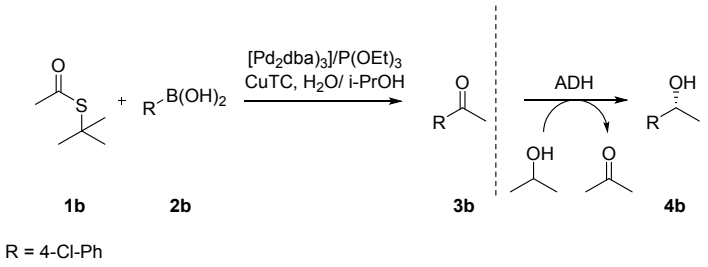
5.2 Coating and baking

The PDMS mixture was evenly distributed (~8 cm diameter) on upside down crystallizing dishes and baked in oven at 65 degrees for 16 hours. The temperature was than increased to 110 °C for 1 hour.

5.3 Delaminating and washing

The coated crystallizing dishes were placed in hexane for 5 min for delamination and the PDMS sheets were soaked twice for 2h in dichloromethane and finally dried in an oven at 110 degree for 2 h.

6 Concurrent chemoenzymatic reaction generating enantiopure alcohols



Chamber A: Liebeskind Srogl coupling chamber

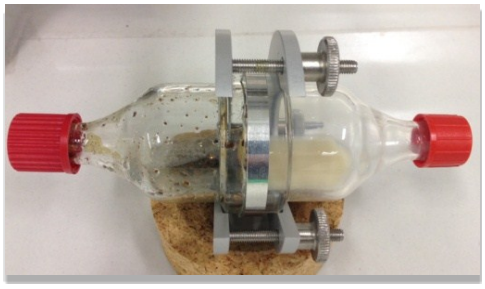
100 mM concentration of **1b**, boronic acid (**2b**, 1.7 equiv.), CuTC (1.6 equiv.), $Pd_2(dba)_3$ (2.5 mol%), $P(OEt)_3$ (20 mol%), was mixed/suspended in H_2O.

Chamber B: Enzymatic chamber

Lyophilized cells of *E. coli* ADH-A (150 mg) respective LK-ADH (200 mg) were rehydrated in Tris HCl buffer (pH 8, 320mM) in a Greiner tube (50 ml) and agitated for 1 h at 30°C with 200 rpm. Before reaction chamber A was filled, 2-propanol (30% v/v) was added into the Greiner tube and mixed.

The PDMS sheet was placed on one of the glass parts of the reactor, the sealing ring was applied and both reactor parts were screwed together holding the PDMS membrane between the two reaction chambers.

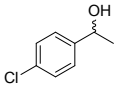
Finally, the enzymatic mixture was transferred into chamber A and the membrane reactor was agitated at 30°C and 200 rpm for 40 h. Out of each reaction chamber 20 µl were taken and the reaction was stopped by quenching with 180 µl AcCN with 11 mM methylbenzoate (in case of 1-(4-fluorophenyl)ethan-1-ol and 1-(4-CF_3-phenyl)ethan-1-ol 11 mM 2-methylanisole) was added. The mixture was vortexed for 1min, centrifuged and analyzed by UPC^2 from Waters according to the general procedure depicted in chapter 1.



6.1 Chemoenzymatic concurrent reaction - Preparative Scale

After full consumption of the starting material was determined by UPC^2-analysis (40h), the chambers of the respective reactor were combined (2 × 2.5 ml) extracted with Et_2O (3 × 10 ml). The combined organic layers were washed with brine, dried over Na_2SO_4 and the solvent was removed. The desired alcohol was purified by column chromatography using DCM (Rf: 0.25). The enatiopure alcohols were obtained in good yield and perfect enationselectivity Table S4. The TBDMS-membrane could be reused after careful treatment upon work-up.

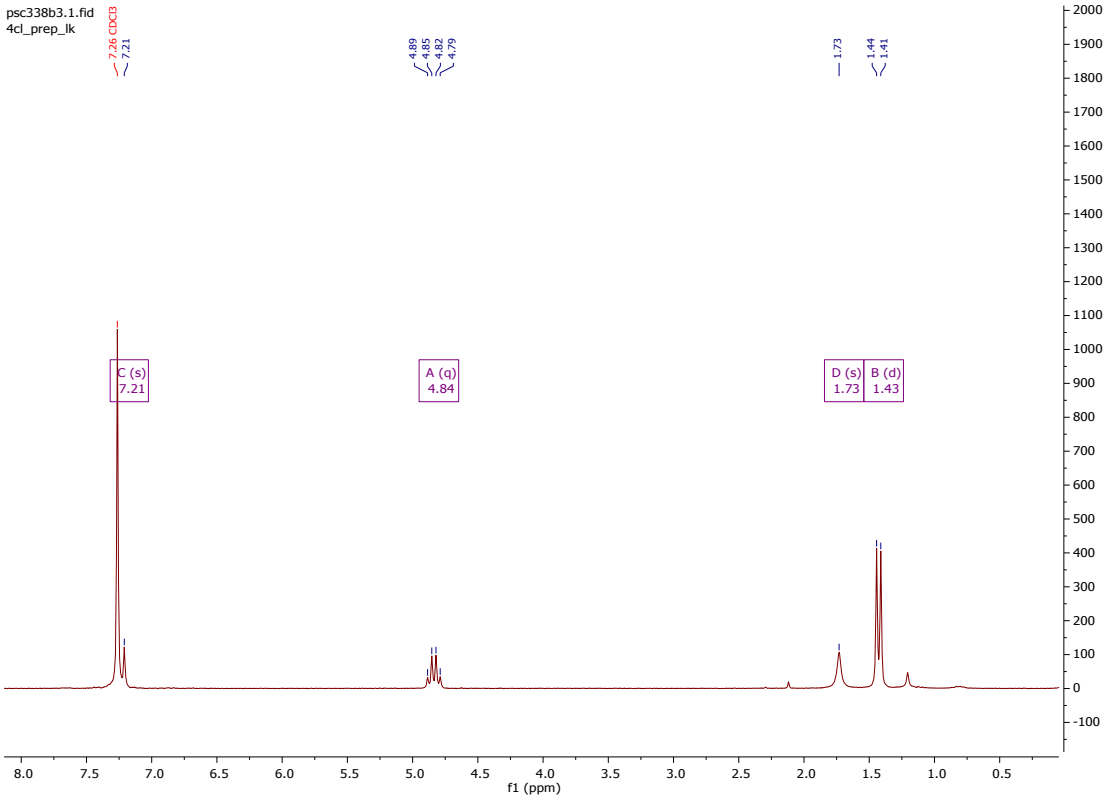
Table S4 Preparative concurrent synthesis of chiral alcohols 4b – (*R*) & (*S*)

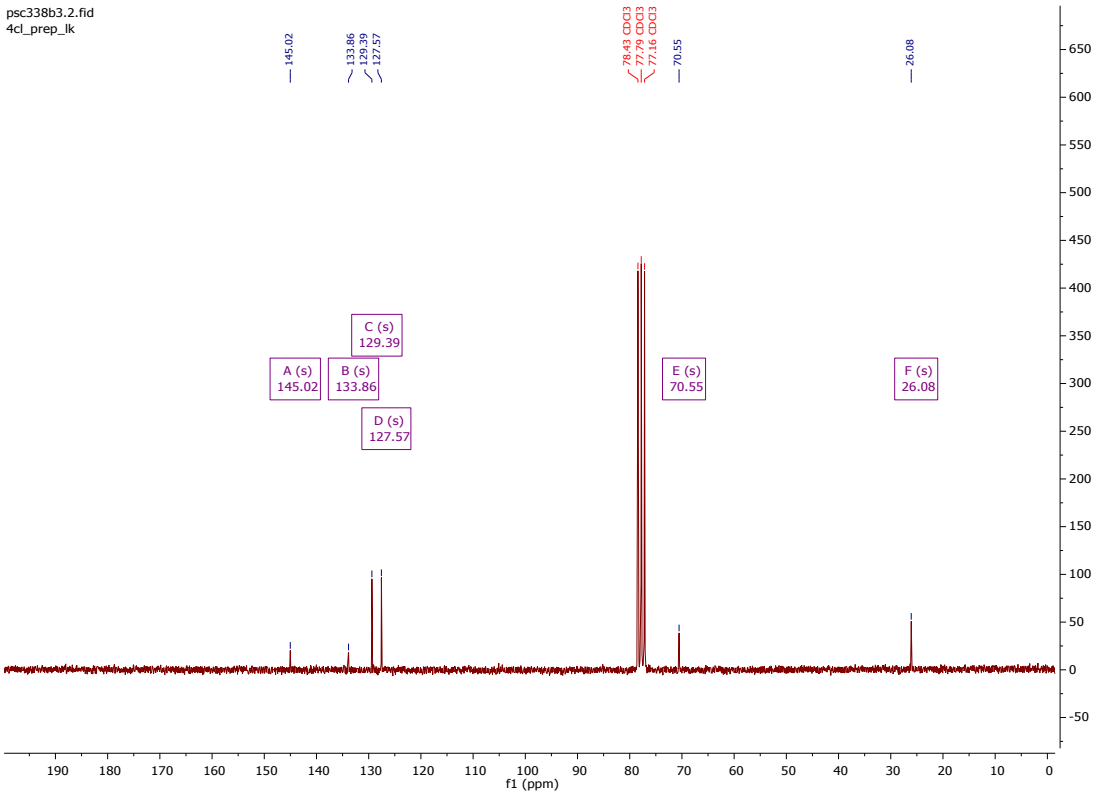
Product 4b	**ADH-A (S)-alcohol Yield [%], 40 h**	**LK-ADH (R)-alcohol Yield [%], 40 h**
	47 (99% ee) 18mg	51 (99% ee) 20mg

6.1.1 8.1 (S)-1-(4-chlorophenyl)ethanol 4b

^{1}H NMR (200 MHz, Chloroform-*d*) δ 1.43 (d, *J* = 6.4 Hz, 3H), 1.73 (s, 1H), 4.84 (q, *J* = 6.5 Hz, 1H), 7.21 (s, 4H).

^{13}C NMR (50 MHz, Chloroform-*d*) δ 26.1, 70.6, 127.6, 129.4, 133.9, 145.0.

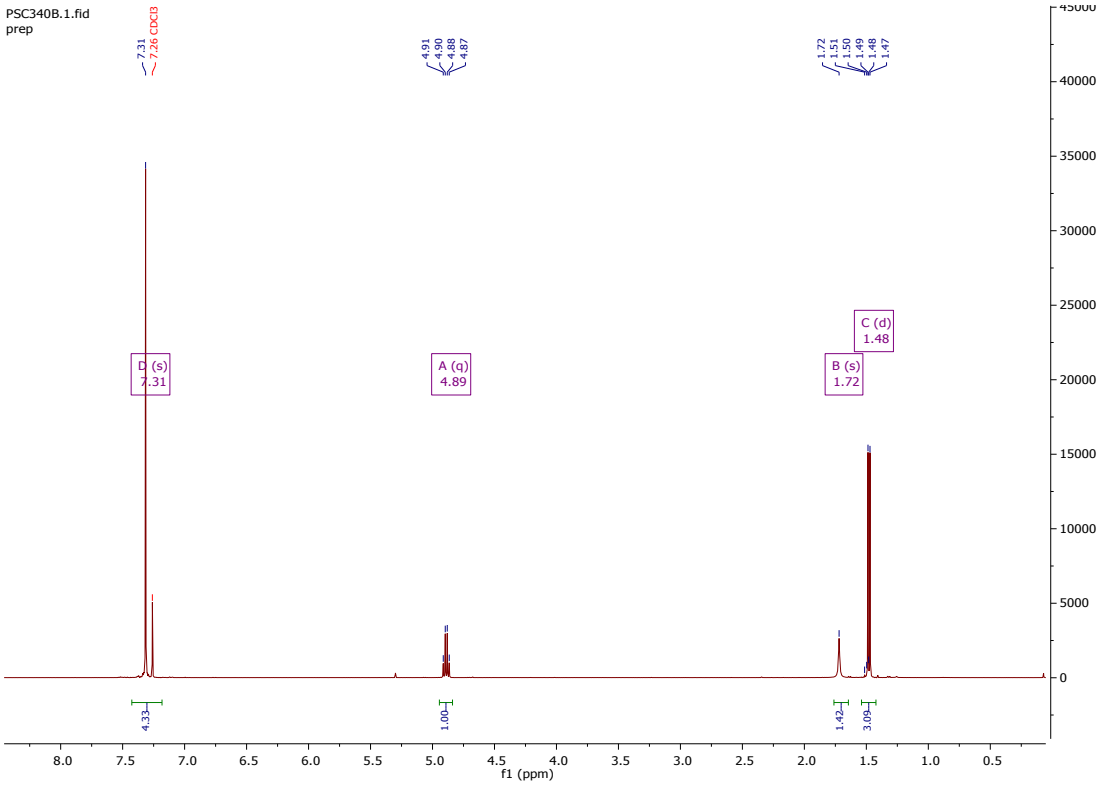


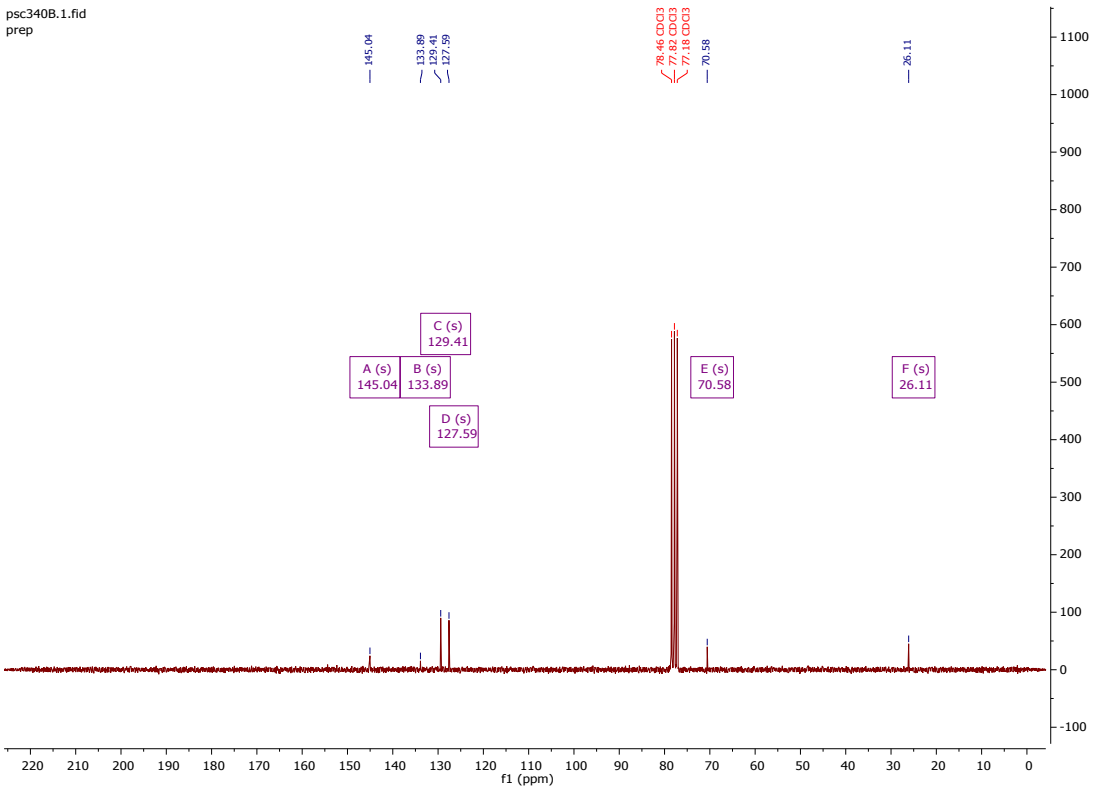


6.1.2 8.2 (R)-1-(4-chlorophenyl)ethanol 4b

^{1}H NMR (400 MHz, Chloroform-*d*) δ 1.48 (d, *J* = 6.5 Hz, 3H), 1.72 (s, 1H), 4.89 (q, *J* = 6.4 Hz, 1H), 7.31 (s, 4H).

^{13}C NMR (400 MHz, Chloroform-*d*) δ 26.1, 70.6, 127.6, 129.4, 133.9, 145.0.





7 References

[1] P. Schaaf, V. Gojic, T. Bayer, F. Rudroff, M. Schnürch, M. D. Mihovilovic, *ChemCatchem* **2018**, *10*, 920-924.
[2] A. L. Miller, N. B. Bowden, *J. Org. Chem.* **2009**, *74*, 4834-4840.