Supporting Information

An Enzymatic 2-Step Cofactor and Co-Product Recycling Cascade towards a Chiral 1,2-Diol. Part I: Cascade Design

Justyna Kulig, Torsten Sehl, Ursula Mackfeld, Wolfgang Wiechert, Martina Pohl, and Dörte Rother*

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– Supplementary Information –

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1. Strategies for the 1-pot 2-step cascade for the synthesis of the 1,2-diol (1*R*,2*R*)-1-phenylpropane-1,2-diol (PPD) with cofactor regeneration and co-product recycling in two different cascade modes: Route A and Route B

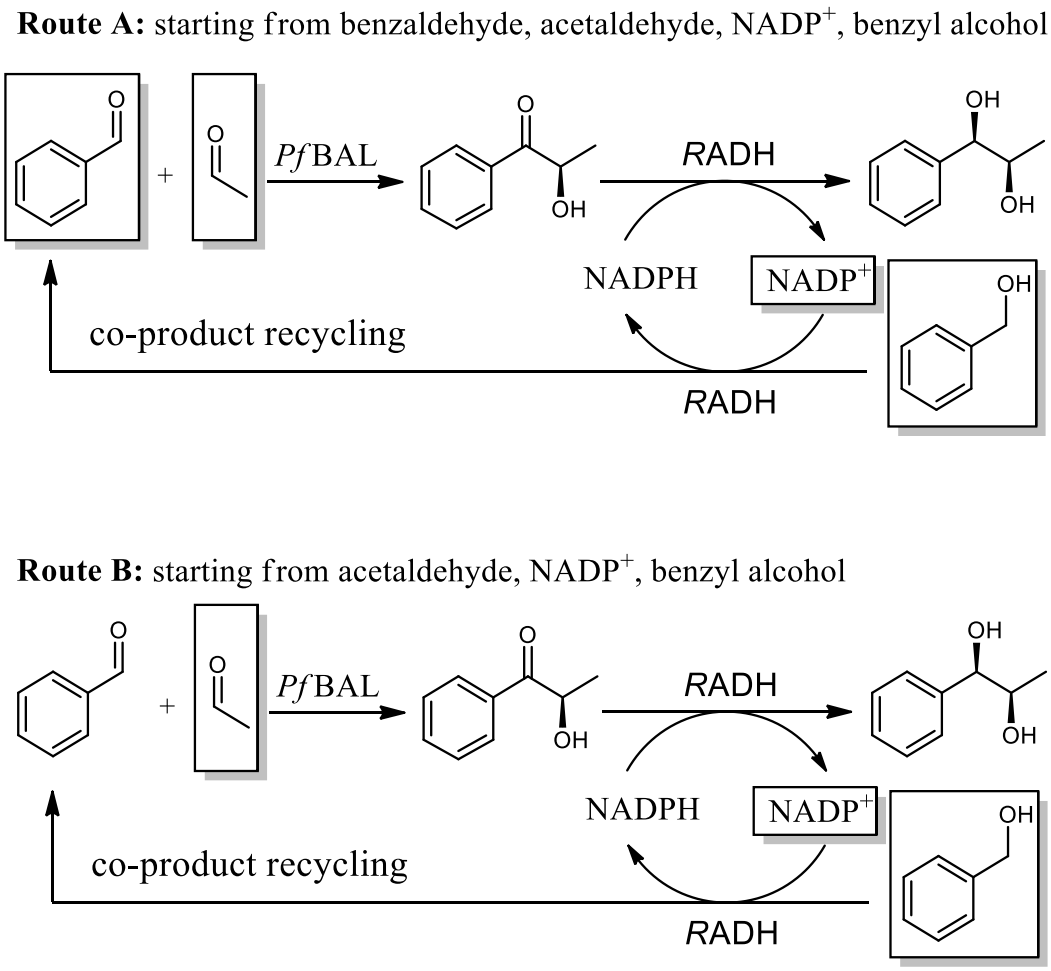


Figure S1. Synthesis of the 1,2-diol (1*R*,2*R*)-1-phenylpropane-1,2-diol (PPD) with cofactor regeneration and co-product recycling in two different cascade modes: Route A and Route B

Two routes for the synthetic cascade reaction with substrate-coupled cofactor regeneration were tested (A and B). They differ in the substrate and co-substrate supply strategy. In all cases, benzyl alcohol was used as the co-substrate. The benzaldehyde formed upon oxidation by RADH is reused in the first step of the cascade. Compounds that were present in the reaction system when the reaction was started (t = 0) are framed. BAL = benzaldehyde lyase from *Pseudomonas fluorescens*, *R*ADH = alcohol dehydrogenase from *Ralstonia* sp.

2. Non-optimized synthesis of (1*R*,2*R*)-1,2-diol using the 1-pot 2-step cascade with cofactor regeneration and co-product recycling

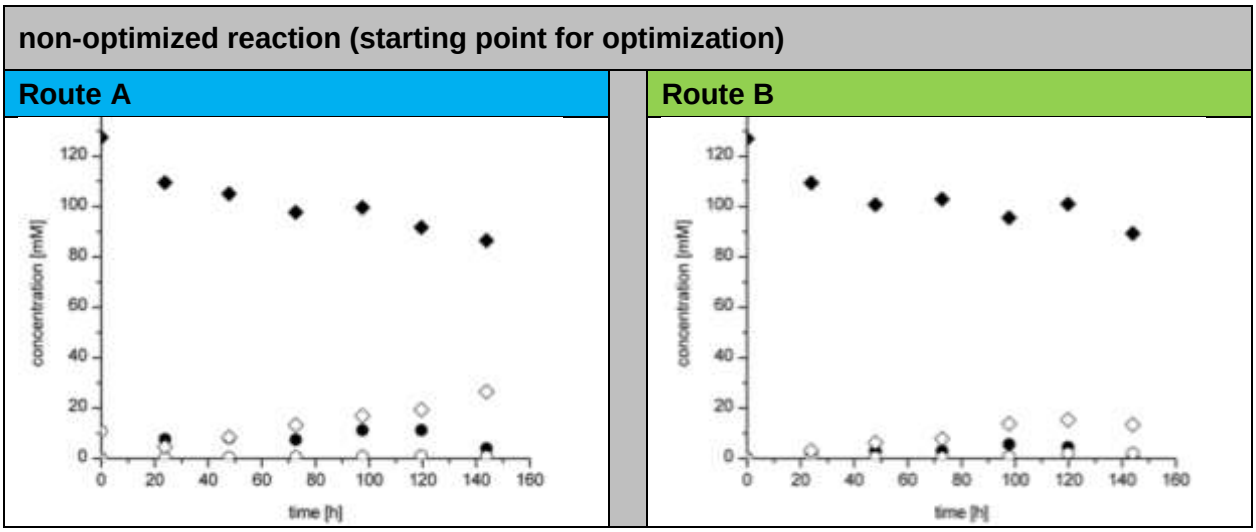


Figure S2. Time-dependent (1*R*,2*R*)-1,2-diol formation with cofactor regeneration and co-product recycling. Here non-optimized "standard" reactions were used.

Reaction conditions: TEA-HCl buffer (50 mM) supplemented with $CaCl_2$ (0.8 mM), $MgSO_4$ (2.5 mM), ThDP (0.15 mM), pH 8.0, $NADP^+$ (0.2 mM), BAL (0.05 mg mL^{-1}), *R*ADH (0.05 mg mL^{-1}). **A.** Route A (Fig. S1): benzaldehyde (10 mM), acetaldehyde (150 mM), benzyl alcohol (120 mM) **B.** Route B (Fig. S1): acetaldehyde (150 mM), benzyl alcohol (120 mM). Reactions were carried out at 20 °C with constant shaking (150 rpm). Samples were taken in defined time intervals.

Symbols: ◆ benzyl alcohol, ○ benzaldehyde, ● (*R*)-2-HPP, ◇ 1,2-diol.

3. Optimisation of the 1-pot 1-step cascade for the synthesis of (1*R*,2*R*)-1,2-diol with cofactor regeneration and co-product recycling

3.1. Varied parameter: $NADP^+$ concentration

Figure S3. Time-dependent (1*R*,2R)-1,2-diol formation with cofactor regeneration and co-product recycling. Here, with varied $NADP^+$ concentrations (0.4-0.8 mM) via route A and B.

Reaction conditions: TEA-HCl buffer (50 mM) supplemented with $CaCl_2$ (0.8 mM), $MgSO_4$ (2.5 mM), ThDP (0.15 mM), pH 8.0, $NADP^+$ (0.2-0.8 mM), BAL (0.05 mg mL^{-1}), RADH (0.10 mg mL^{-1}). **A.** Route A: benzaldehyde (10 mM), acetaldehyde (150 mM), benzyl alcohol (120 mM) **B.** Route B: acetaldehyde (150 mM), benzyl alcohol (120 mM). Reactions were carried out at 20 °C with constant shaking (150 rpm). Samples were taken in defined intervals.

Symbols: ◆ benzyl alcohol, ○ benzaldehyde, ● (*R*)-2-HPP, ◇ 1,2-diol.

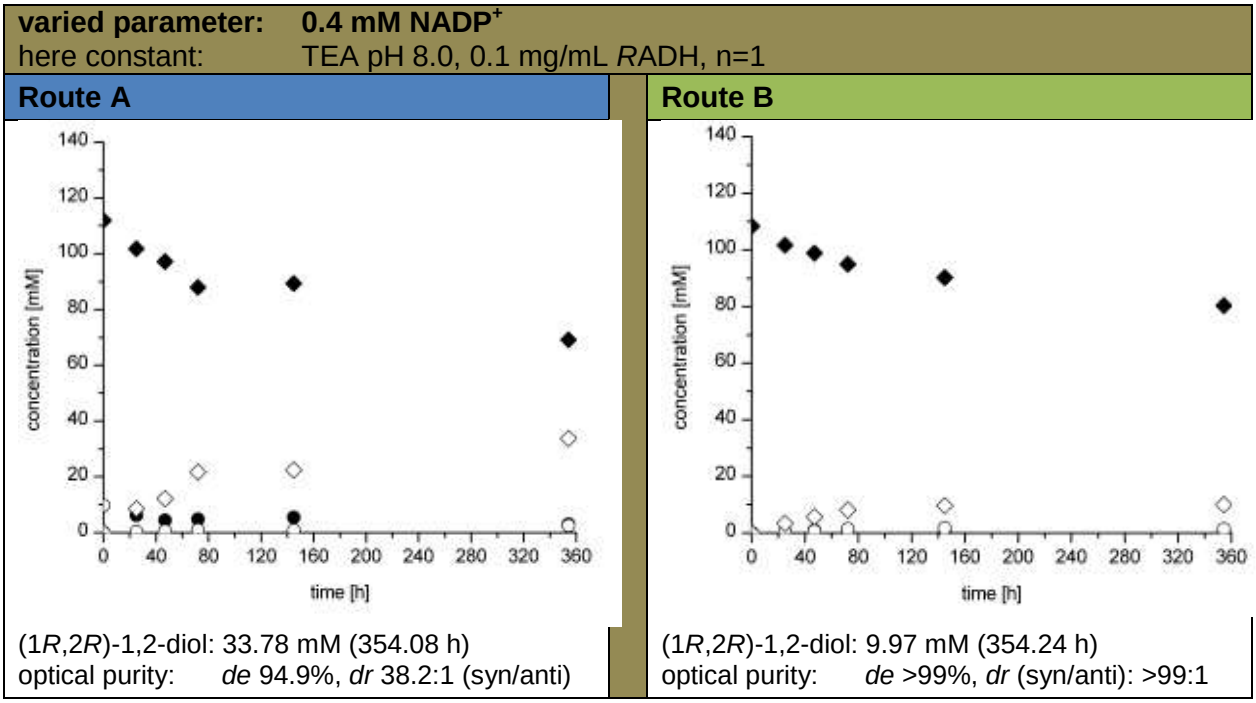
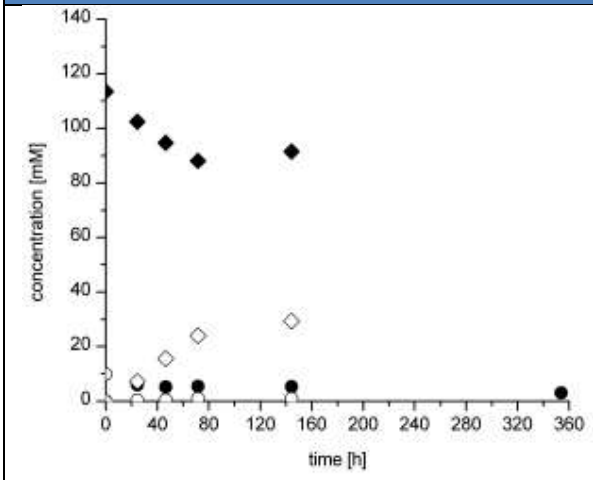


Figure S3. continued from above

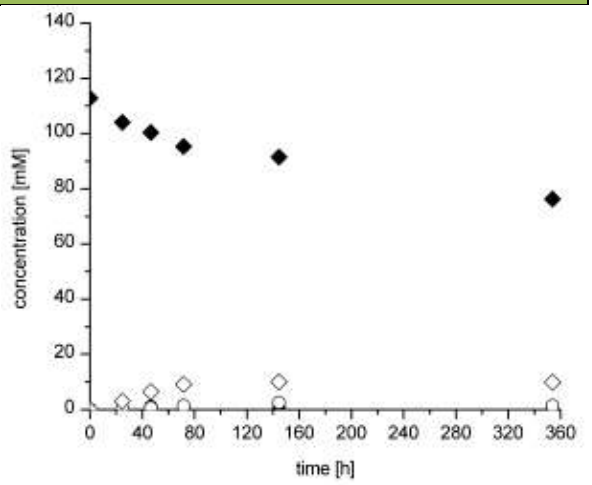
varied parameter: **0.6 mM NADP$^+$**
here constant: TEA pH 8.0, 0.1 mg/mL *R*ADH, n=1

Route A



(1*R*,2*R*)-1,2-diol: 29.33 mM (144.41 h)
optical purity: *de* 95.7%, *dr* 45.5:1 (syn/anti)

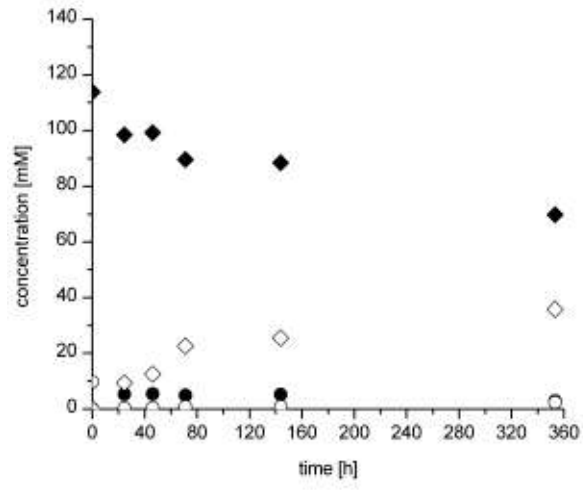
Route B



(1*R*,2*R*)-1,2-diol: 10.07 mM (144.58 h)
optical purity: *de* >99%, *dr* (syn/anti): >99:1

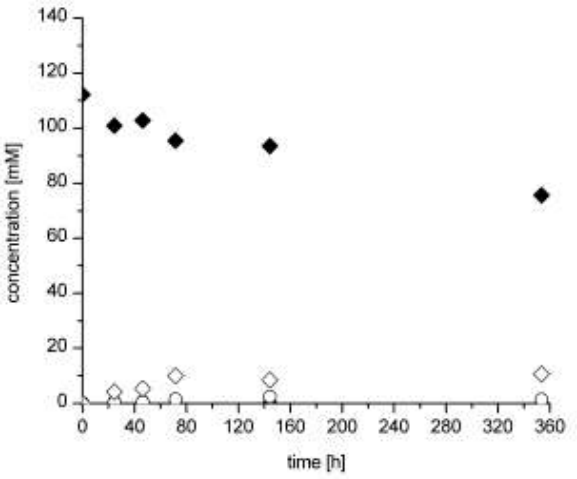
varied parameter: **0.8 mM NADP$^+$**
here constant: TEA pH 8.0, 0.1 mg/mL *R*ADH, n=1

Route A



(1*R*,2*R*)-1,2-diol: 35.76 mM (353.24 h)
optical purity: *de* 94.8%, *dr* 37.7:1 (syn/anti)

Route B



(1*R*,2*R*)-1,2-diol: 10.69 mM (353.66 h)
optical purity: *de* >99%, *dr* (syn/anti): >99:1

Figure S3. Time-dependent (1*R*,2R)-1,2-diol formation with cofactor regeneration and co-product recycling. Here, with varied NADP$^+$ concentrations (0.4-0.8 mM) via route A and B.

Reaction conditions: TEA-HCl buffer (50 mM) supplemented with $CaCl_2$ (0.8 mM), $MgSO_4$ (2.5 mM), ThDP (0.15 mM), pH 8.0, NADP$^+$ (0.2-0.8 mM), BAL (0.05 mg mL^{-1}), *R*ADH (0.10 mg mL^{-1}). **A.** Route A: benzaldehyde (10 mM), acetaldehyde (150 mM), benzyl alcohol (120 mM) **B.** Route B: acetaldehyde (150 mM), benzyl alcohol (120 mM). Reactions were carried out at 20 °C with constant shaking (150 rpm). Samples were taken in defined intervals.

Symbols: ◆ benzyl alcohol, ○ benzaldehyde, ● (*R*)-2-HPP, ◇ 1,2-diol.

3.2. Varied parameter: pH value and buffer

A) Route A

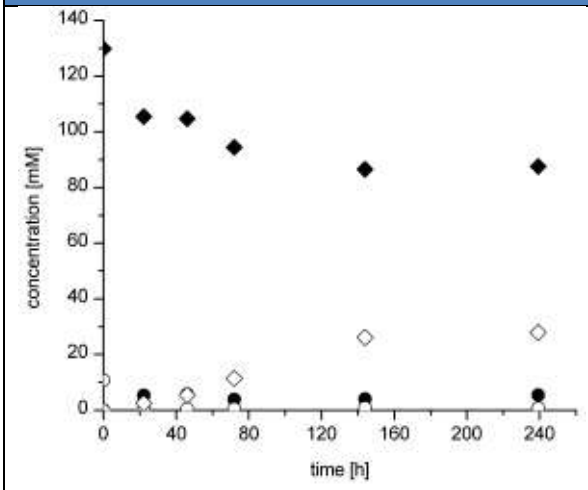
Figure S4.A Time-dependent (1*R*,2R)-1,2-diol formation with cofactor regeneration and co-product recycling. Here, with varied pH value and buffer (TEA pH 8.0-9-0 and TRIS-HCl pH 9.0) via route A and B.

Reaction conditions: buffer (50 mM) with $CaCl_2$ (0.8 mM), $MgSO_4$ (2.5 mM), ThDP (0.15 mM), TEA pH 8.0-9-0 or TRIS-HCl pH 9.0, $NADP^+$ (0.2 mM), BAL (0.05 mg mL^{-1}), *R*ADH (0.10 mg mL^{-1}). **A.** Route A: benzaldehyde (10 mM), acetaldehyde (150 mM), benzyl alcohol (120 mM) **B.** Route B: acetaldehyde (150 mM), benzyl alcohol (120 mM). Reactions were carried out at 20 °C with constant shaking (150 rpm). Samples were taken in defined intervals.

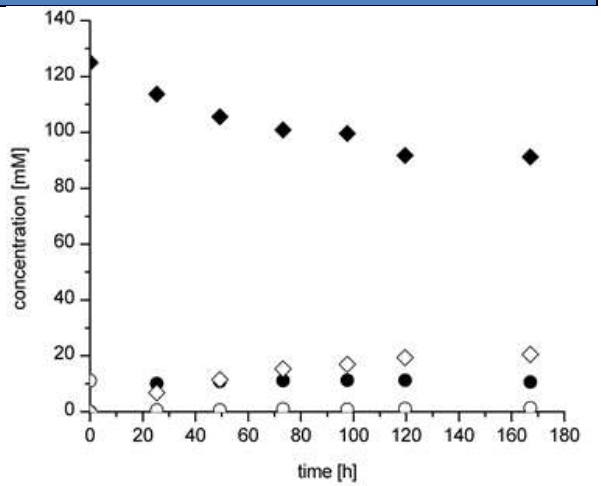
Symbols: ◆ benzyl alcohol, ○ benzaldehyde, ● (*R*)-2-HPP, ◇ 1,2-diol.

varied parameter:	**TEA pH 8.0**
here constant:	0.2 mM $NADP^+$, 0.1 mg/mL RADH, n=2

Route A



(1*R*,2*R*)-1,2-diol: 27.98 mM (239.17 h)
optical purity: *de* 95.6%, *dr* 44.9:1 (syn/anti)

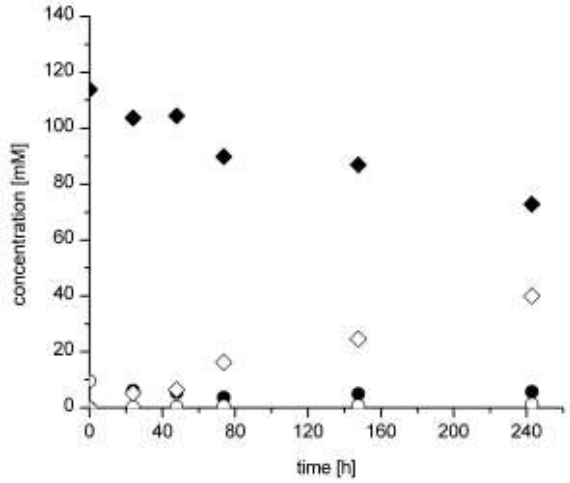


(1*R*,2*R*)-1,2-diol: 20.55 mM (167.08 h)
optical purity: *de* >99%, *ee* >99%, *dr* (syn/anti): >99:1

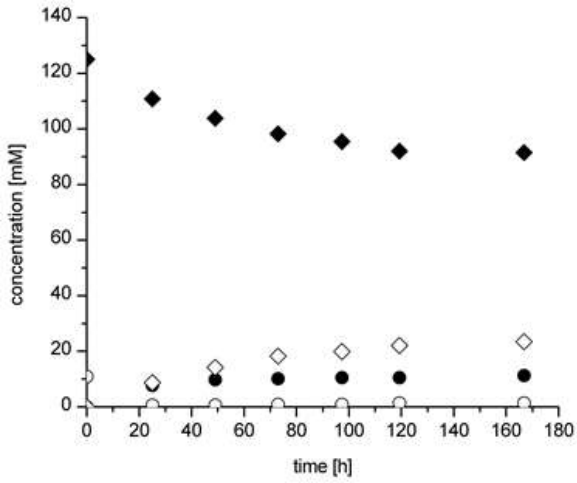
Figure S4.A continued from above

varied parameter:	**TEA pH 8.5**
here constant:	0.2 mM $NADP^+$, 0.1 mg/mL RADH, n=2

Route A



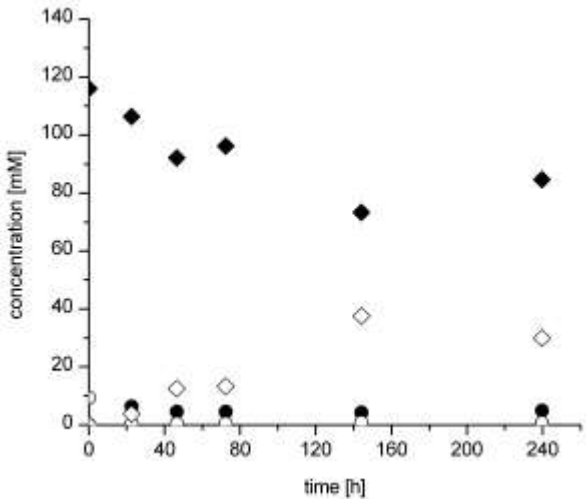
(1*R*,2*R*)-1,2-diol: 40.00 mM (242.92 h)
optical purity: *de* 96.6%, *dr* 58.3:1 (syn/anti)



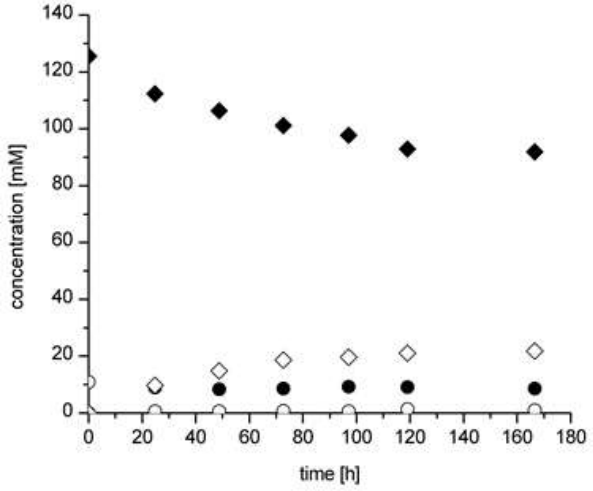
(1*R*,2*R*)-1,2-diol: 23.52 mM (166.83 h)
optical purity: *de* > 99%, *ee* > 99%, *dr* (syn/anti): –

varied parameter:	**TEA pH 9.0**
here constant:	**0.2 mM $NADP^+$, 0.1 mg/mL RADH, n=2**

Route A



(1*R*,2*R*)-1,2-diol: 37.56 mM (144.17 h)
optical purity: *de* 97.9%, *dr* 92.9:1 (syn/anti)



(1*R*,2*R*)-1,2-diol: 21.80 mM (166.58 h)
optical purity: *de* > 99%, *ee* > 99%, *dr* (syn/anti): –

Figure S4.A Time-dependent (1*R*,2*R*)-1,2-diol formation with cofactor regeneration and co-product recycling. Here, with varied pH value and buffer (TEA pH 8.0-9-0 and TRIS-HCl pH 9.0) via route A and B.

Reaction conditions: buffer (50 mM) with $CaCl_2$ (0.8 mM), $MgSO_4$ (2.5 mM), ThDP (0.15 mM), TEA pH 8.0-9-0 or TRIS-HCl pH 9.0, $NADP^+$ (0.2 mM), BAL (0.05 mg mL^{-1}), *R*ADH (0.10 mg mL^{-1}). **A.** Route A: benzaldehyde (10 mM), acetaldehyde (150 mM), benzyl alcohol (120 mM) **B.** Route B: acetaldehyde (150 mM), benzyl alcohol (120 mM). Reactions were carried out at 20 °C with constant shaking (150 rpm). Samples were taken in defined intervals.

Symbols: ◆ benzyl alcohol, ○ benzaldehyde, ● (*R*)-2-HPP, ◇ 1,2-diol.

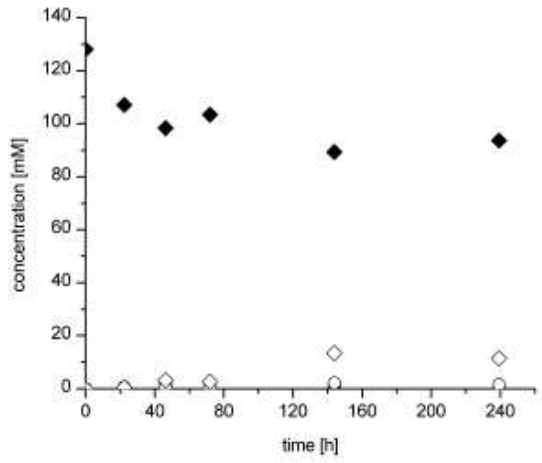
B) Route B

Figure S4.B Time-dependent (1*R*,2R)-1,2-diol formation with cofactor regeneration and co-product recycling. Here, with varied pH value and buffer (TEA pH 8.0-9-0 and TRIS-HCl pH 9.0) via route A and B.

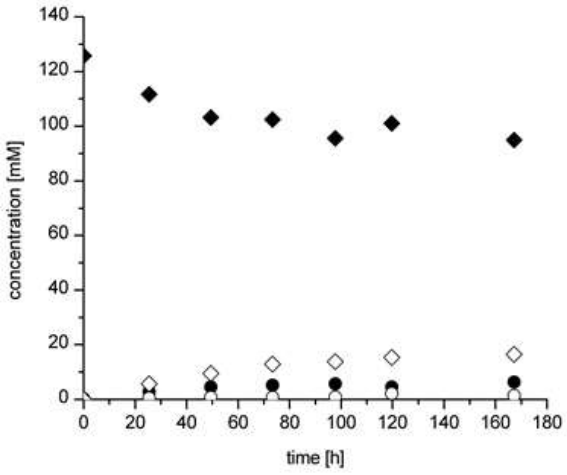
Reaction conditions: buffer (50 mM) with $CaCl_2$ (0.8 mM), $MgSO_4$ (2.5 mM), ThDP (0.15 mM), TEA pH 8.0-9-0 or TRIS-HCl pH 9.0, $NADP^+$ (0.2 mM), BAL (0.05 mg mL^{-1}), *R*ADH (0.10 mg mL^{-1}). **A.** Route A: benzaldehyde (10 mM), acetaldehyde (150 mM), benzyl alcohol (120 mM) **B.** Route B: acetaldehyde (150 mM), benzyl alcohol (120 mM). Reactions were carried out at 20 °C with constant shaking (150 rpm). Samples were taken in defined intervals.

Symbols: ◆ benzyl alcohol, ○ benzaldehyde, ● (*R*)-2-HPP, ◇ 1,2-diol.

varied parameter:	**TEA pH 8.0**
here constant:	0.2 mM $NADP^+$, 0.1 mg/mL RADH, n=2
Route B	



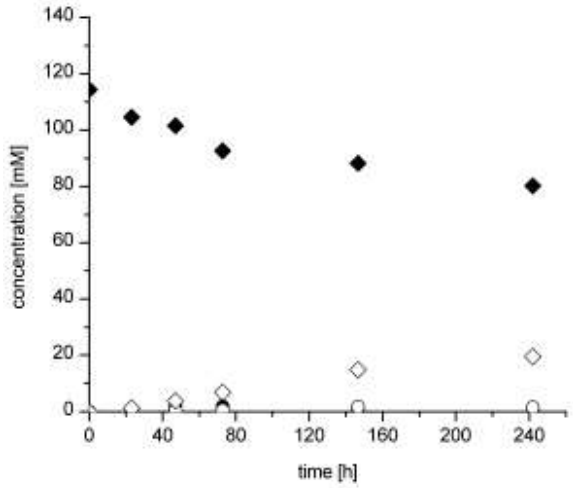
(1*R*,2*R*)-1,2-diol: 13.43 mM (143.92 h)
optical purity: *de* >99%, *ee* >99%, *dr* (syn/anti): >99:1



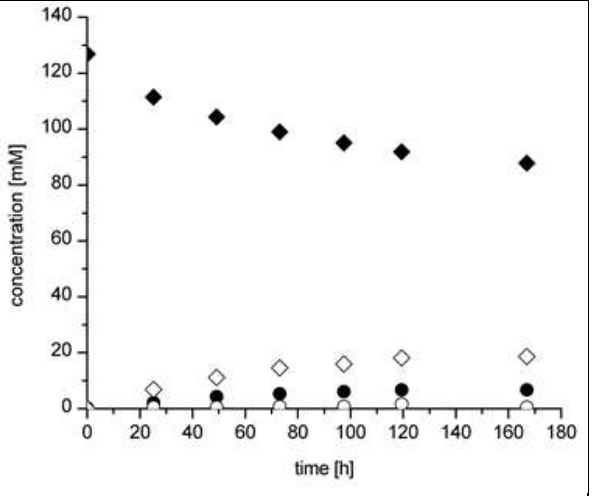
(1*R*,2*R*)-1,2-diol: 16.49 mM (167.25 h)
optical purity: *de* >99%, *ee* >99%, *dr* (syn/anti): >99:1

Figure S4.B continued from above

varied parameter:	**TEA pH 8.5**
here constant:	0.2 mM $NADP^+$, 0.1 mg/mL RADH, n=2
Route B	

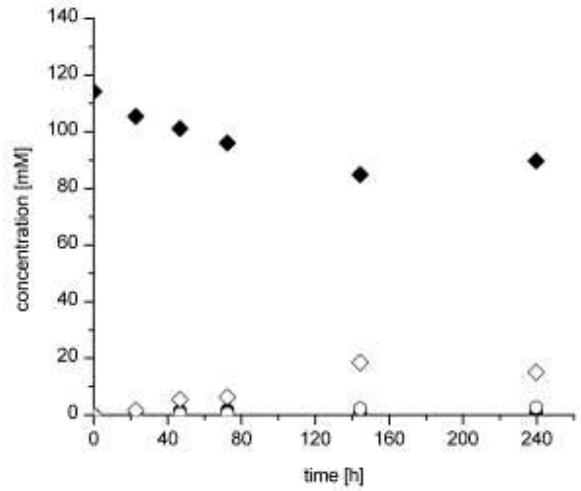


(1*R*,2*R*)-1,2-diol: 19.49 mM (242.00 h)
optical purity: *de* >99%, *ee* >99%,
dr (syn/anti): >99:1

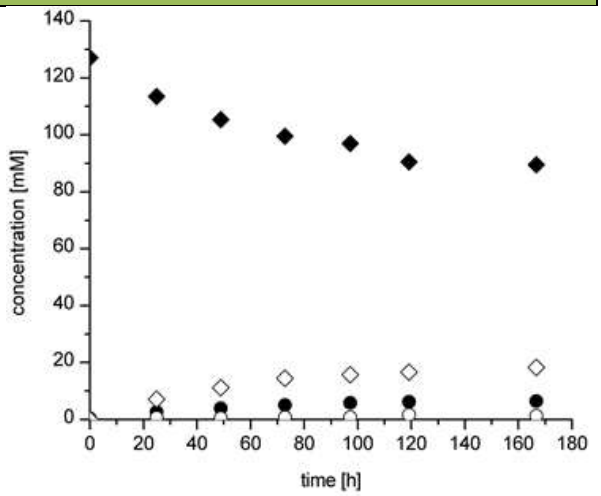


(1*R*,2*R*)-1,2-diol: 18.64 mM (166.92 h)
optical purity: *de* >99%, *ee* >99%,
dr (syn/anti): >99:1

varied parameter:	**TEA pH 9.0**
here constant:	0.2 mM $NADP^+$, 0.1 mg/mL RADH, n=2
Route B	



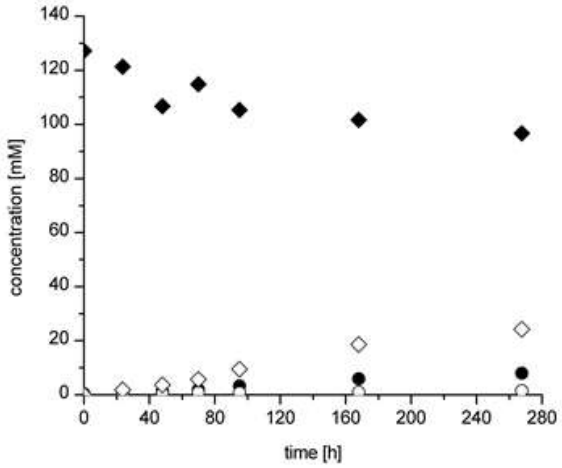
(1*R*,2*R*)-1,2-diol: 18.53 mM (144.34 h)
optical purity: *de* >99%, *ee* >99%,
dr (syn/anti): >99:1



(1*R*,2*R*)-1,2-diol: 18.27 mM (166.67 h)
optical purity: *de* >99%, *ee* >99%,
dr (syn/anti): >99:1

Figure S4.B continued from above

varied parameter:	**TRIS pH 9.0**
here constant:	0.2 mM $NADP^+$, 0.1 mg/mL RADH, n=1
Route B	



(1*R*,2*R*)-1,2-diol: 24.24 mM (267.59 h)
optical purity: *de* > 99%, *ee* > 99%, *dr* (syn/anti): >99:1

Figure S4.B Time-dependent (1*R*,2*R*)-1,2-diol formation with cofactor regeneration and co-product recycling. Here, with varied pH value and buffer (TEA pH 8.0-9-0 and TRIS-HCl pH 9.0) via route A and B.

Reaction conditions: buffer (50 mM) with $CaCl_2$ (0.8 mM), $MgSO_4$ (2.5 mM), ThDP (0.15 mM), TEA pH 8.0-9-0 or TRIS-HCl pH 9.0, $NADP^+$ (0.2 mM), BAL (0.05 mg mL^{-1}), *R*ADH (0.10 mg mL^{-1}). **A.** Route A: benzaldehyde (10 mM), acetaldehyde (150 mM), benzyl alcohol (120 mM) **B.** Route B: acetaldehyde (150 mM), benzyl alcohol (120 mM). Reactions were carried out at 20 °C with constant shaking (150 rpm). Samples were taken in defined intervals.

Symbols: ◆ benzyl alcohol, ○ benzaldehyde, ● (*R*)-2-HPP, ◇ 1,2-diol.

3.3. Varied parameter: RADH concentration

Figure S5. Time-dependent (1*R*,2*R*)-1,2-diol formation with cofactor regeneration and co-product recycling. Here, with varied RADH concentrations (0.10-2.00 mg/mL) via route A and B.

Reaction conditions: TRIS-HCl buffer (50 mM) supplemented with $CaCl_2$ (0.8 mM), $MgSO_4$ (2.5 mM), ThDP (0.15 mM), pH 9.0, $NADP^+$ (0.2 mM), BAL (0.05 mg mL^{-1}), RADH (0.10-2.00 mg mL^{-1}). **A.** Route A: benzaldehyde (10 mM), acetaldehyde (150 mM), benzyl alcohol (120 mM) **B.** Route B: acetaldehyde (150 mM), benzyl alcohol (120 mM). Reactions were carried out at 20 °C with constant shaking (150 rpm). Samples were taken in defined intervals.

Symbols: ◆ benzyl alcohol, ○ benzaldehyde, ● (*R*)-2-HPP, ◇ 1,2-diol.

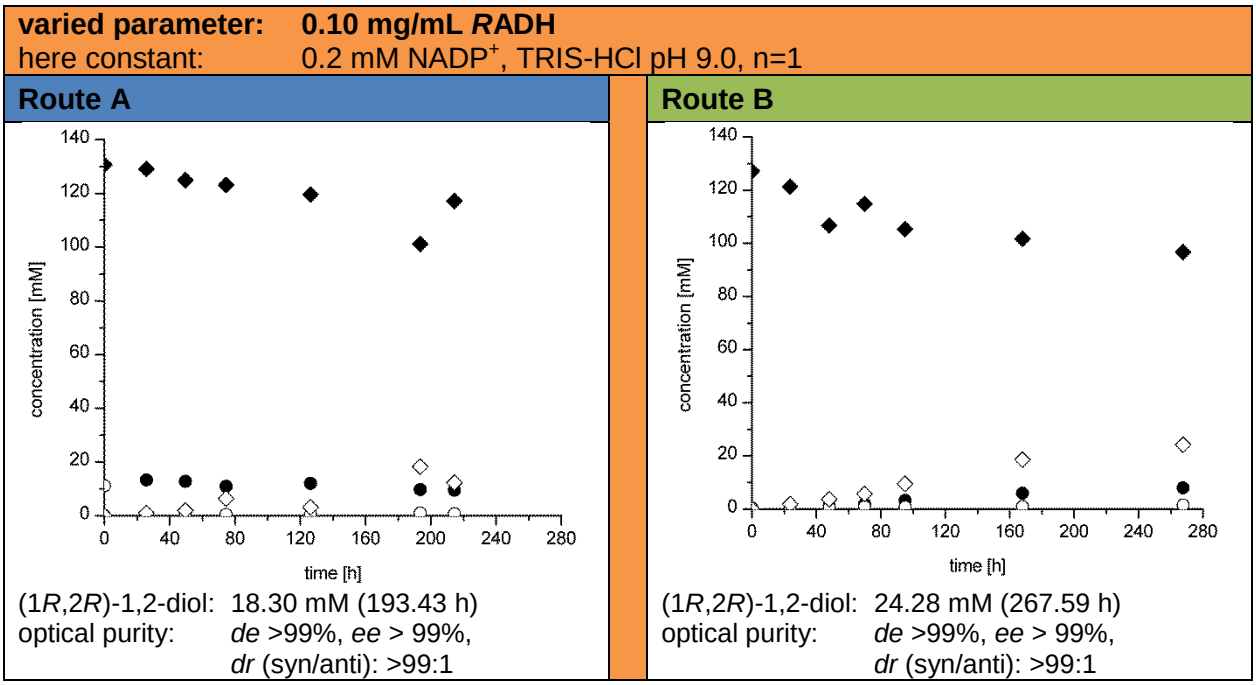
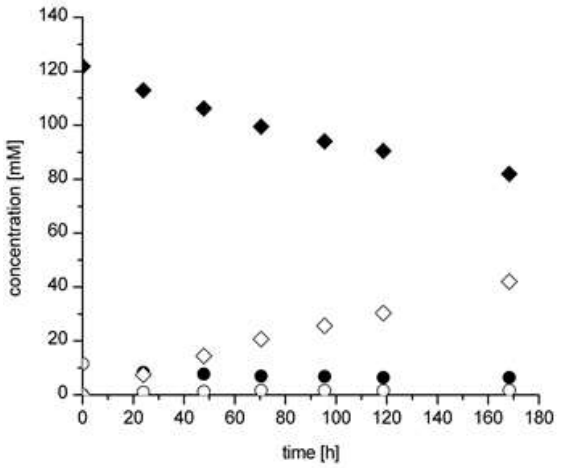


Figure S5. continued from above

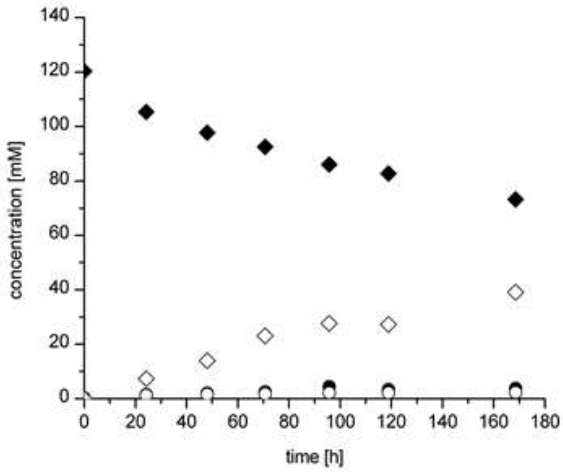
varied parameter: **0.24 mg/mL *R*ADH**
here constant: 0.2 mM $NADP^+$, TRIS-HCl pH 9.0, n=1

Route A



(1*R*,2*R*)-1,2-diol: 41.97 mM (168.33 h)
optical purity: *de* 97.1%, *ee* > 99%, *dr* (syn/anti): 69.1:1

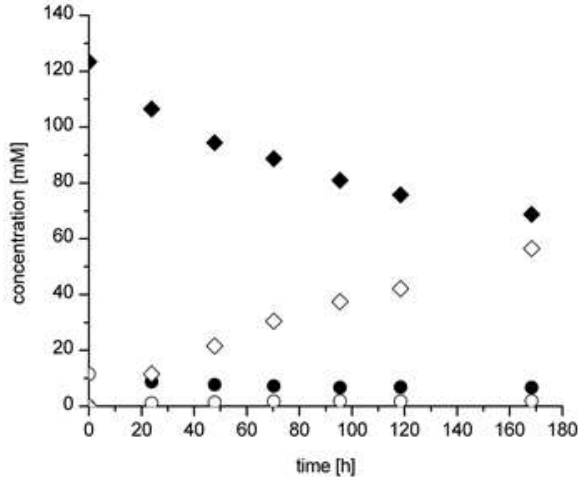
Route B



(1*R*,2*R*)-1,2-diol: 39.03 mM (168.58 h)
optical purity: *de* 98.0%, *ee* >99%, dr (syn/anti): 97.8:1

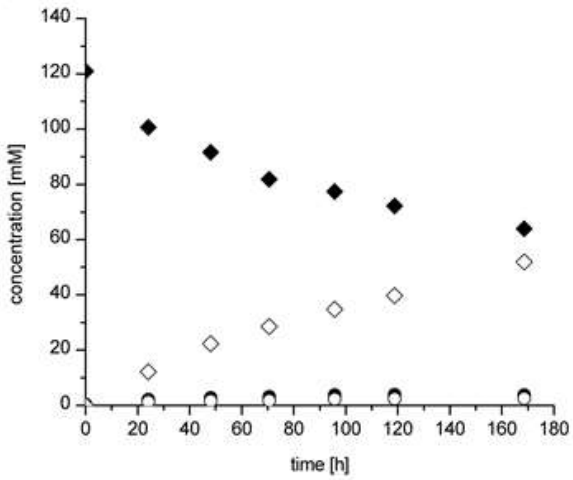
varied parameter: **0.33 mg/mL *R*ADH**
here constant: 0.2 mM $NADP^+$, TRIS-HCl pH 9.0, n=1

Route A



(1*R*,2*R*)-1,2-diol: 56.49 mM (168.24 h)
optical purity: *de* 97.6%, *ee* >99%, *dr* (syn/anti): 81.8:1

Route B

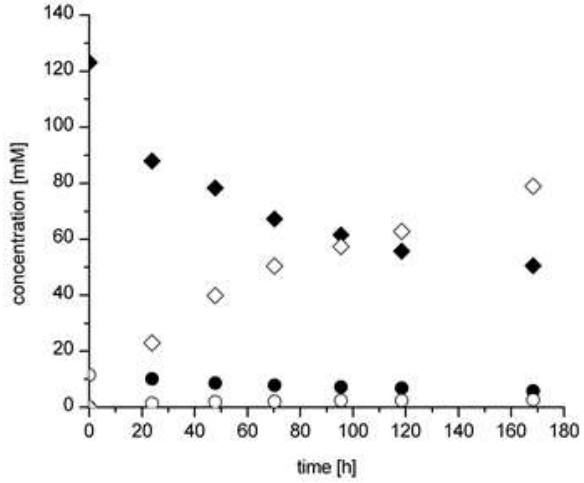


(1*R*,2*R*)-1,2-diol: 51.87 mM (168.49 h)
optical purity: *de* 98.2%, *ee* >99%, *dr* (syn/anti): >99:1

Figure S5. continued from above

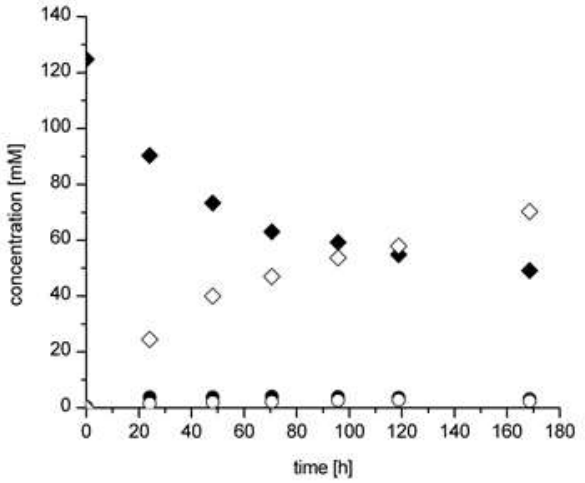
varied parameter: **0.64 mg/mL *R*ADH**
here constant: **0.2 mM NADP$^+$, TRIS-HCl pH 9.0, n=1**

Route A



(1*R*,2*R*)-1,2-diol: 78.89 mM (168.24 h)
optical purity: *de* 96.7%, *ee* >99%,
dr (syn/anti): 60.5:1

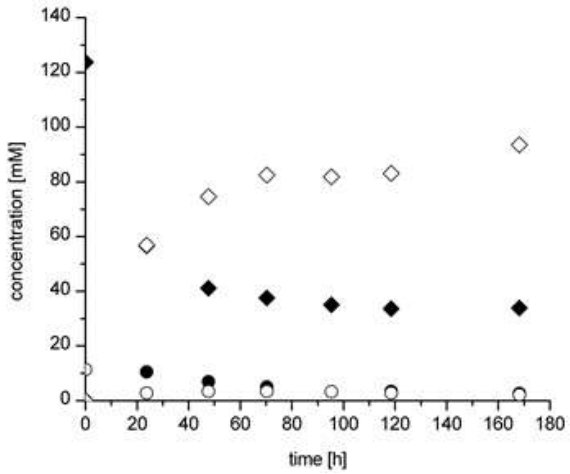
Route B



(1*R*,2*R*)-1,2-diol: 70.26 mM (168.49 h)
optical purity: *de* 96.2%, *ee* >99%,
dr (syn/anti): 52.1:1

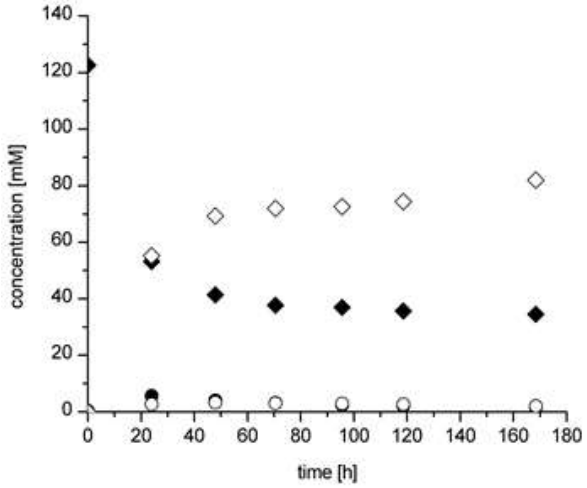
varied parameter: **2.00 mg/mL *R*ADH**
here constant: 0.2 mM NADP$^+$, TRIS-HCl pH 9.0, n=1

Route A



(1*R*,2*R*)-1,2-diol: 93.49 mM (168.16 h)
optical purity: *de* 91.3%, *ee* >99%,
dr (syn/anti): 22.1:1

Route B



(1*R*,2*R*)-1,2-diol: 82.03 mM (168.41 h)
optical purity: *de* 90.2%, *ee* >99%,
dr (syn/anti): 19.3:1

Figure S5. Time-dependent (1*R*,2*R*)-1,2-diol formation with cofactor regeneration and co-product recycling. Here, with varied *R*ADH concentrations (0.10-2.00 mg/mL) via route A and B.

Reaction conditions: TRIS-HCl buffer (50 mM) supplemented with $CaCl_2$ (0.8 mM), $MgSO_4$ (2.5 mM), ThDP (0.15 mM), pH 9.0, NADP$^+$ (0.2 mM), BAL (0.05 mg mL^{-1}), RADH (0.10-2.00 mg mL^{-1}). **A.** Route A: benzaldehyde (10 mM), acetaldehyde (150 mM), benzyl alcohol (120 mM) **B.** Route B: acetaldehyde (150 mM), benzyl alcohol (120 mM). Reactions were carried out at 20 °C with constant shaking (150 rpm). Samples were taken in defined intervals.
Symbols: ◆ benzyl alcohol, ○ benzaldehyde, ● (*R*)-2-HPP, ◇ 1,2-diol.

4. Equilibrium computation for the cascade reaction using substrate-coupled cofactor regeneration

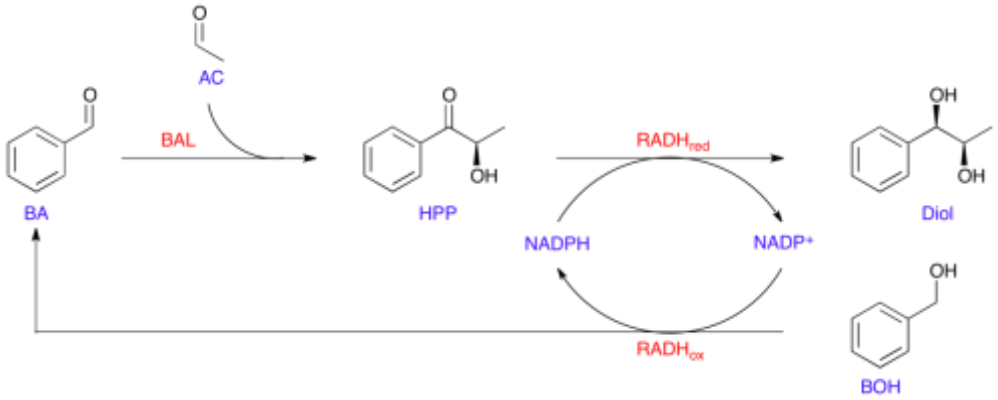


Fig. S6. 2-Step enzymatic synthesis of 1,2-diol with *in situ* co-product removal.

BAL = benzaldehyde lyase from *Pseudomonas fluorescens*, *R*ADH$_{red}$ = reduction catalysed by an alcohol dehydrogenase from *Ralstonia* sp., *R*ADH$_{ox}$ = oxidation catalysed by an alcohol dehydrogenase from *Ralstonia* sp., BA = benzaldehyde, BOH = benzyl alcohol, AC = acetaldehyde, HPP = (*R*)-2-hydroxy-1-propiophenone.

Table S1. Equilibrium concentration data used for the calculation of reaction equilibrium constants.

BAL: starting conditions and resulting concentration as published earlier
***R*ADH**: denotes the sum reaction of *R*ADH$_{red}$ and *R*ADH$_{ox}$ (see text).

	BAL	*R*ADH
benzaldehyde (BA)	0.6 mM	9.5 mM
acetaldehyde (AC)	10.6 mM	·
(*R*)-2-HPP	19.4 mM	0.5 mM
1,2-Diol	·	9.5 mM
benzyl alcohol (BOH)	·	90.5 mM

From the data given in Table S1 the reaction equilibrium constant for the BAL reaction (Figure S6) is calculated as:

$$K_{BAL}^{eq} = \frac{[HPP]_{eq}}{[AC]_{eq} \cdot [BA]_{eq}} = 6.4\ mM^{-1} \qquad (4)$$

In equilibrium, the other two reactions can be considered as one single sum reaction because their rates must be equal and the cofactors NADPH, NADP$^+$ both behave stoichiometrically and thermodynamically neutral. The equilibrium constant of the sum reaction is (cf. Table S1):

$$K_{RADH}^{eq} = \frac{[Diol]_{eq} \cdot [BA]_{eq}}{[HPP]_{eq} \cdot [BOH]_{eq}} = 2.0 \qquad (5)$$

The balance equations describing the dynamics of all substance concentrations are given by:

$$
\begin{aligned}
\dot{[BA]} &= -r_{BAL} + r_{RADH_{ox}} \\
\dot{[AC]} &= -r_{BAL} \\
\dot{[HPP]} &= r_{BAL} - r_{RADH_{red}} \\
\dot{[Diol]} &= r_{RADH_{red}} \\
\dot{[BOH]} &= -r_{RADH_{ox}} \\
\dot{[NADPH]} &= -r_{RADH_{red}} + r_{RADH_{ox}} \\
\dot{[NADP^+]} &= r_{RADH_{red}} - r_{RADH_{ox}}
\end{aligned}
\tag{6}
$$

The reaction system equilibrium does not depend on the type of kinetics of the reaction steps. For this reason, any kinetic terms can be assumed without influencing the equilibrium. Thus, simple reversible mass action laws can be used for computing the reaction equilibrium:

$$
\begin{aligned}
r_{BAL} &= k^+_{BAL} \times [BA] \times [AC] - k^-_{BAL} \times [HPP] \\
r_{RADH_{red}} &= k^+_{RADH_{red}} \times [HPP] \times [NADPH] - k^-_{RADH_{red}} \times [Diol] \times [NADP^+] \\
r_{RADH_{ox}} &= k^+_{RADH_{ox}} \times [BOH] \times [NADP^+] - k^-_{RADH_{ox}} \times [BA] \times [NADPH]
\end{aligned}
\tag{7}
$$

The kinetic parameters must be chosen to reproduce the equilibrium constants (notice that $[NADPH], [NADP^+]$ cancel out):

$$
\begin{aligned}
&\frac{k^+_{BAL}}{k^-_{BAL}} = K^{eq}_{BAL} \\
&\frac{k^+_{RADH_{red}}}{k^-_{RADH_{red}}} \times \frac{k^+_{RADH_{ox}}}{k^-_{RADH_{ox}}} = K^{eq}_{RADH_{red}} \times K^{eq}_{RADH_{ox}} = K^{eq}_{RADH}
\end{aligned}
\tag{8}
$$

Any choice of the 6 reaction parameters

$$k^+_{BAL}, k^-_{BAL}, k^+_{RADH_{red}}, k^-_{RADH_{red}}, k^+_{RADH_{ox}}, k^-_{RADH_{ox}}$$

which is consistent with Eqs. (8) will lead to a suitable reaction equilibrium. To obtain the theoretical reaction equilibrium corresponding to a specific experiment, also the initial concentrations

$$[BA]_0,[AC]_0,[HPP]_0,[BOH]_0,[Diol]_0,[NADPH]_0,[NADP^+]_0$$

must be supplied. Implementing equations (6), (7) in MATLAB and using the built-in numerical differential equation solver ode45 reaction equilibria can now be computed. The differential equation solver implicitly cares for the following four independent mass conservation relations which can be derived from the reaction stoichiometry:

$$\begin{aligned} [NADPH]+[NADP^+] &= \text{const.} \\ [BA]+[HPP]+[Diol]+[BOH] &= \text{const.} \\ [AC]+[HPP]+[Diol] &= \text{const.} \\ [Diol]+[BOH]+[NADPH] &= \text{const.} \end{aligned} \qquad (9)$$

These conservation equations can be used for plausibility checking of the computed result.

Table S2. Theoretical overall conversions of 1,2-diol (related to benzyl alcohol and HPP) calculated for **routes A and B** (see main text). Green highlighted is the added starting material.

Initial values implemented for the simulation:

Route A: $[BA]_0$=10, $[AC]_0$=150, $[BAOH]_0$=120, $[NADP^+]_0$=0.20, $[HPP]_0$=10, $[Diol]_0$=0, $K_{BAL}^{eq} = 6.4\,mM^{-1}$,$K_{RADH}^{eq} = 2.0$

Route B: $[BA]_0$=0, $[AC]_0$=150, $[BAOH]_0$=120, $[NADP^+]_0$=0.20, $[HPP]_0$=10, $[Diol]_0$=0, $K_{BAL}^{eq} = 6.4\,mM^{-1}$,$K_{RADH}^{eq} = 2.0$

Route A

	concentration [mM]						
	BA	AC	HPP	Diol	BOH	NADPH	$NADP^+$
Initial	10.00	150.00	0.00	0.00	120.00	0.00	0.20
Equilibrium	0.08	20.53	10.09	119.37	0.45	0.17	0.03

Theoretical conversion: **~90 %** (91.8%)

Route B

	concentration [mM]						
	BA	AC	HPP	Diol	BOH	NADPH	$NADP^+$
Initial	0.00	150.00	0.00	0.00	120.00	0.00	0.20
Equilibrium	0.00	30.56	0.20	119.24	0.56	0.20	0.00

Theoretical conversion: **>99 %** (99.4%)

To this end the simulation is run long enough for establishing a steady state. Figure S7 gives an example of a simulation run. Notice that the simulation is not meant as a proper model for the true process but just as a computational vehicle to compute the theoretical conversion rates. The computed rates are shown in Table S2.

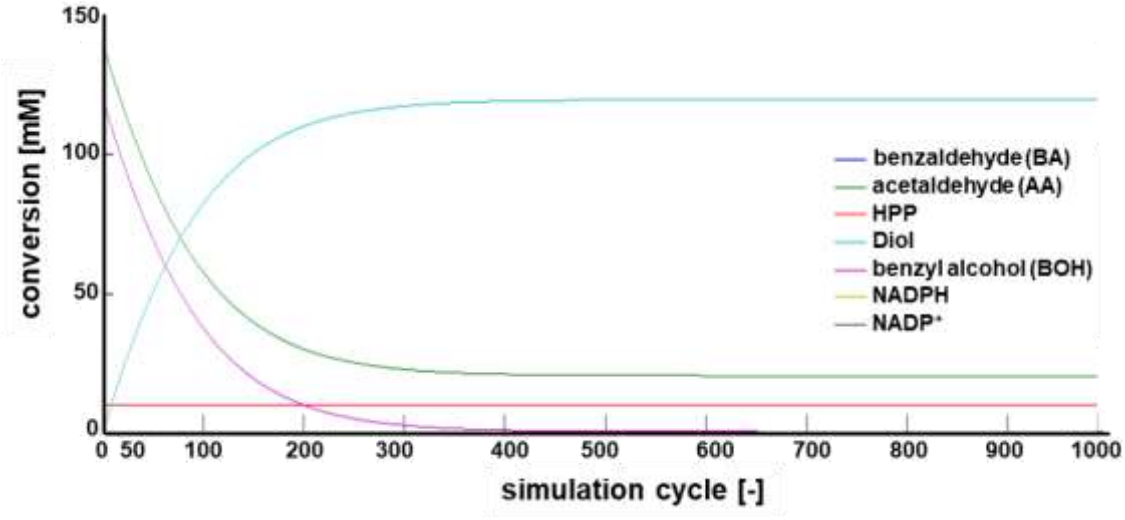
Figure S7. Simulation of the theoretical overall conversion for route A and route B.

Initial values implemented for the simulation:

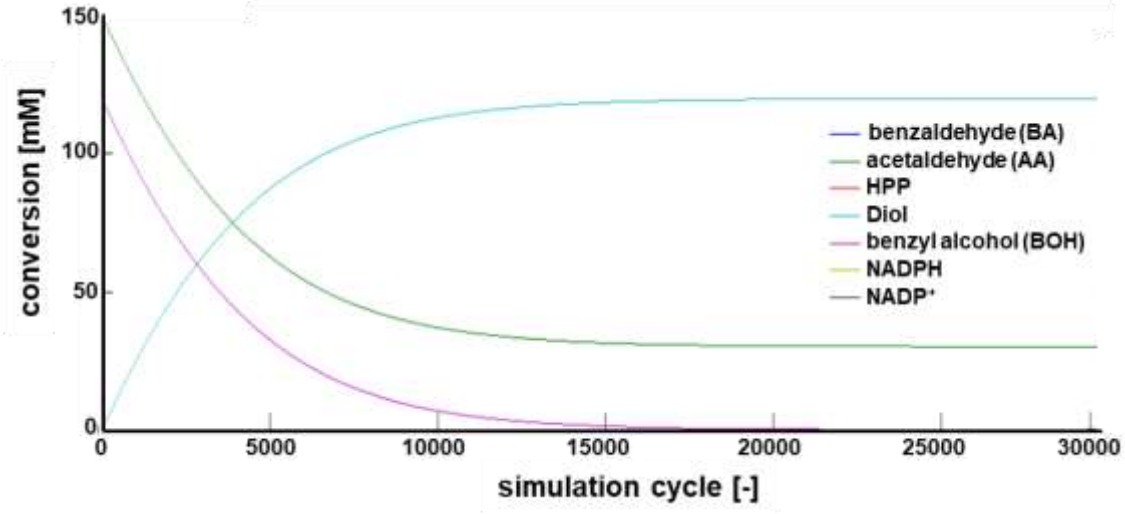
Route A: $[BA]_0$=10, $[AC]_0$=150, $[BAOH]_0$=120, $[NADP^+]_0$=0.20, $[HPP]_0$=10, $[Diol]_0$=0, $K_{BAL}^{eq} = 6.4\ mM^{-1}$,$K_{RADH}^{eq} = 2.0$

Route B: $[BA]_0$=0, $[AC]_0$=150, $[BAOH]_0$=120, $[NADP^+]_0$=0.20, $[HPP]_0$=10, $[Diol]_0$=0, $K_{BAL}^{eq} = 6.4\ mM^{-1}$, $K_{RADH}^{eq} = 2.0$.

Route A



Route B



5. Product isolation and characterization

5.1 Synthesis in preparative scale and protocol for product isolation

A preparative scale synthesis was performed in order to proof product quality after product isolation, however it was not optimized with respect to optimal yields. Thus, a reaction was set-up in a scale of 20 mL containing 50 mM Tris-HCl (pH 9) with 0.8 mM $CaCl_2$, 2.5 mM $MgCl_2$, 0,1 mM ThDP, 120 mM benzyl alcohol, 10 mM benzaldehyde, 150 mM acetaldehyde, 0.2 mM $NADP^+$, as well as 0.64 mg/mL *R*ADH and 0.05 mg/mL *Pf*BAL. The reaction was incubated for 114 h at 20 °C in a glass flask.

The solution was filtrated using an ultrafiltration membrane (Amicon, YM10 membran) with a cut-off of 10 KDa in order to separate the proteins from the reaction solution. Afterwards, the clear solution was extracted 3x20 mL with ethyl acetate. The combined organic fractions were dried with magnesium sulfate and ethyl acetate was evaporated using reduced pressure. 80.2 mg crude product, corresponding to a yield of 22.0 %, could be obtained.

Chromatographic separation was performed using a column filled with 8 g silica gel, which was flushed by petrol ether (PE) and ethyl acetate (EE) in a ration of PE:EE = 3:1. The product was eluted using a step gradient of 60 mL PE:EE = 3:1 followed by 40 mL PE:EE = 1:1 and 40 mL EE. During elution 3 mL fraction were collected. Fractions 26-32 contained the pure product and were combined and evaporated using reduced pressure. 43.0 mg white crystals, corresponding to a yield of 11.7 %, could be obtained.

5.2 Product identification and purity determination

The product was analysed by gas chromatography (as demonstrated within the main article) and by nuclear magnetic resonance spectroscopy in order to verify the product identity and purity.

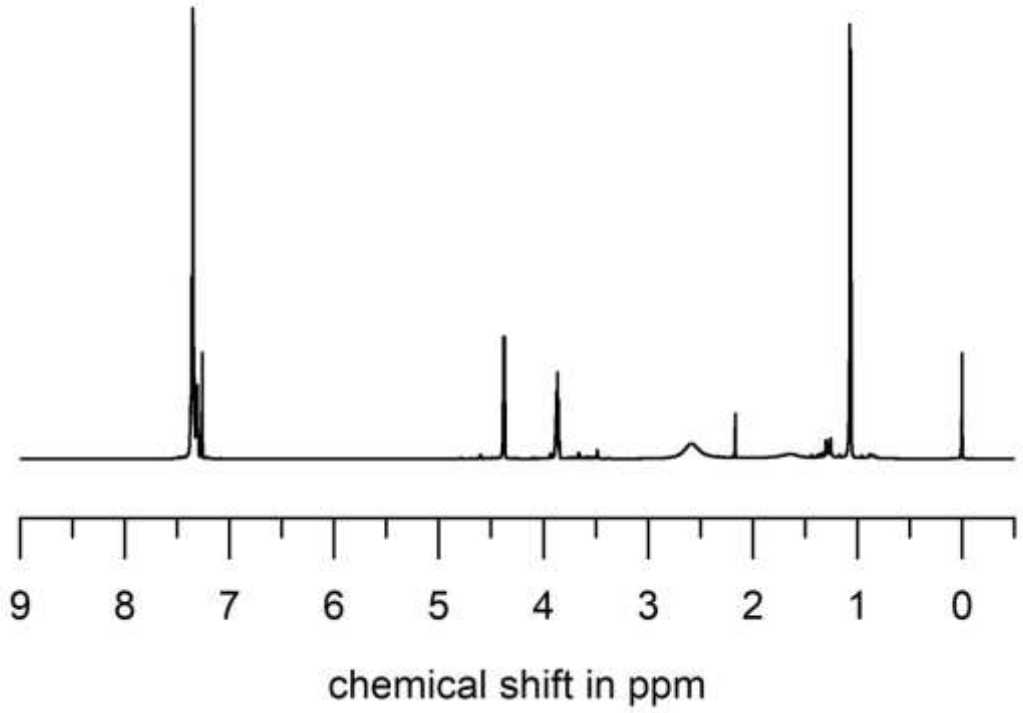
Identification by NMR

1H-NMR (600 MHz, $CDCl_3$). 1.07 (d, $^3J_{1,2}$ = 6.3 Hz, 3H, CH_3), 2.59 (brs 2H, OH); 3.87 (dq, 1H, $^3J_{2,1}$ = 6.3 Hz, 3J2,3 = 7.3 Hz, C_2H), 4.38 (d, $^3J_{3,2}$ = 7.3 Hz, 1H, C_3H), 7.30-7.37 (m, 5H, arom.-H); solvent signal at 7.26.

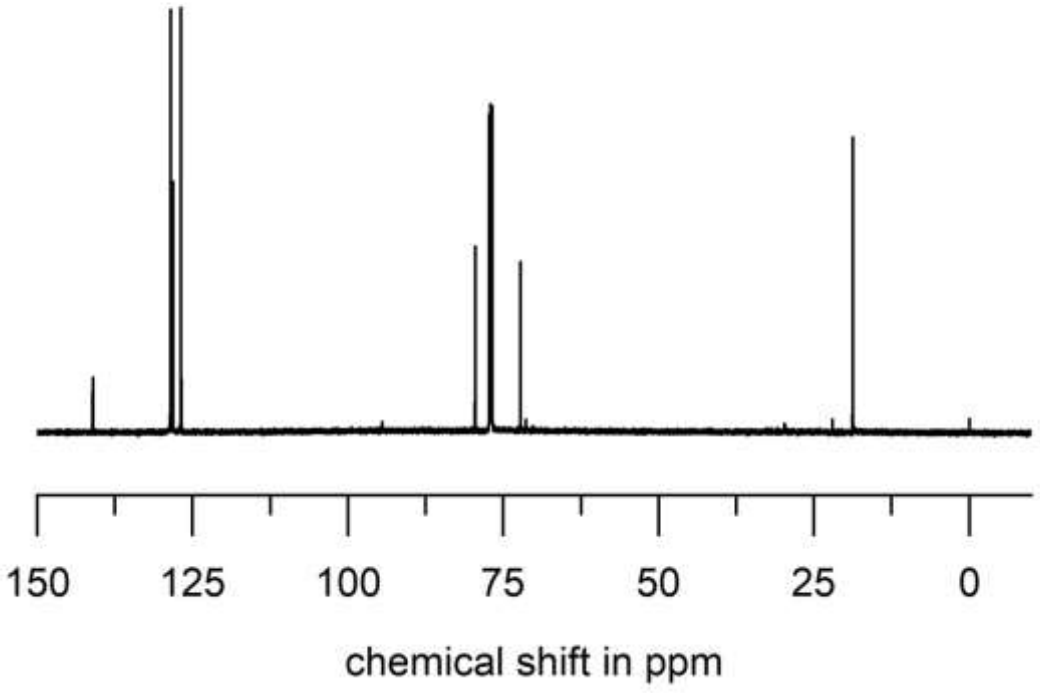
13C-NMR (600 MHz, $CDCl_3$). 18.8 (C-3), 72.2 (C2), 79.5 (C1), 126.8 (arom-CH), 128.2 (arom-CH), 128.5 (arom-CH), 141.1 (arom-CH); three solvent signals at 76.8, 77.0, 77.2.

The spectra match the results for previously published data for (1*R*,2*R*)-1-phenylpropane-1,2-diol. (see: http://www.rsc.org/suppdata/gc/c4/c4gc00010b/c4gc00010b1.pdf) Thus, the product was identified as 1-phenylpropane-1,2-diol by NMR.

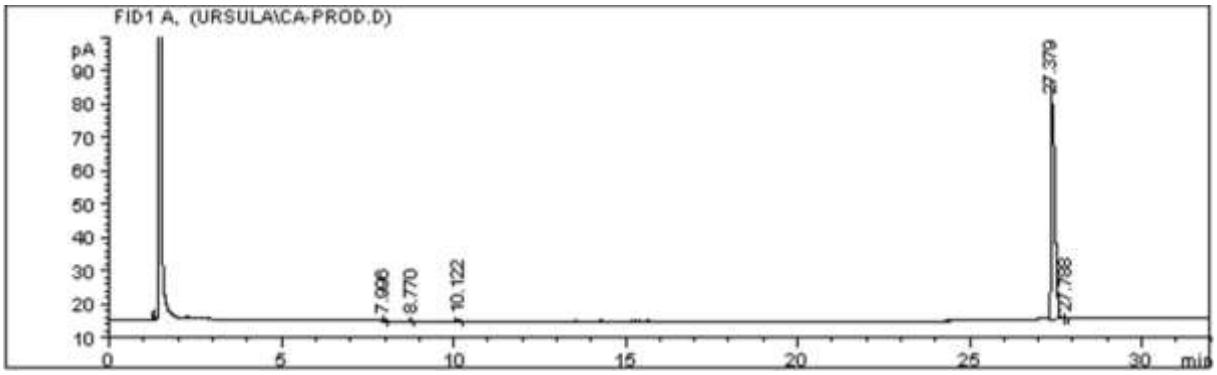
1H-NMR



13C-NMR



Purity according to gas chromatography: 99.4 % (area percent)



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	7.996	PB	0.0324	3.98676e-1	1.69771e-1	0.06999
2	8.770	PB	0.0428	3.48725e-1	1.03433e-1	0.06122
3	10.122	PB	0.0630	1.76762	3.75026e-1	0.31033
4	27.379	VB	0.0955	566.19360	70.99857	99.40440
5	27.788	BV	0.0515	8.77410e-1	2.30138e-1	0.15404
Totals :				569.58604	71.87693	