Electronic Supplementary Information

Deracemisation of profenol core by combining a laccase/TEMPO-mediated oxidation and an alcohol dehydrogenase-catalysed dynamic kinetic resolution

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I. General

***Materials*.** Racemic 2-phenyl-1-propanol, 2-phenylpropionaldehyde, and 2-phenylpropionic acid are commercially available and were obtained from Sigma Aldrich. Laccase from *Trametes versicolor* was also purchased from Sigma Aldrich. *Myceliophthora thermophila* laccase was obtained from Novozymes. *Cerrena unicolor* laccase and *Trametes* sp. were supplied by CLEA Technologies. *Pleurotus ostreatus* laccase was kindly donated by Lentikats. Evo-1.1.200 was purchased from Evocatal. LBADH was obtained from Codexis. ADHs from *Rhodococcus ruber* (ADH-A), *Thermoanaerobium* sp. (ADH-T), *Sphingobium yanoikuyae* (SyADH) and *Ralstonia* sp. (RasADH) were overexpressed following the methodology previously described.[1] HLADH was obtained from Prof. Martina Pohl (Forschungszentrum Jülich GmbH, Germany). All other reagents were obtained from commercial sources and used as received unless otherwise indicated.

***General experimental information*.** Oxidation reactions using the laccase from *Trametes versicolor*/TEMPO catalytic system were performed in a Erlenmeyer flask, open to air and in an orbital shaker.

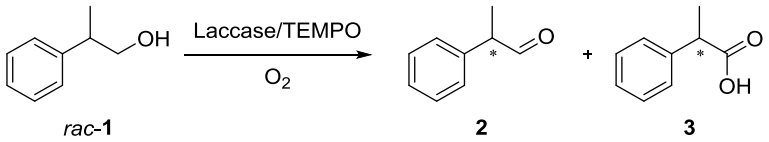
NMR spectra were recorded on a Bruker DPX 300 MHz or AV400 MHz spectrometer. All chemical shifts (δ) are given in parts per million (ppm) and referenced to the residual solvent signal as internal. Gas chromatography (GC) analyses were performed on a Agilent 7820 A GC chromatograph equipped with a FID detector. High performance liquid chromatography (HPLC) analyses were carried out in a Hewlett Packard 1100 chromatograph UV detector at 210 nm. Thin-layer chromatography (TLC) was conducted with Merck Silica Gel 60 F_{254} precoated plates and visualised with UV and potassium permanganate stain. Column chromatography was performed using Merck Silica Gel 60 (230-400 mesh).

In addition to those specified above, the following abbreviations, designations and formulas are used throughout the Supporting Information: DCM= dichloromethane; Na_2SO_4= sodium sulfate; EtOAc = ethyl acetate; TEMPO: 2,2,6,6-tetramethyl-1-piperidinyloxyl radical; EtOH: ethanol; 2-PrOH: 2-propanol; $MgCl_2$: magnesium chloride.

II. Oxidation of 2-phenyl-1-propanol (1) using different laccases

In a 25 mL erlenmeyer flask, to a solution of alcohol **1** (20 mg, 0.14 mmol) in acetate buffer 50 mM pH 5.5 (4.5 mL), was added TEMPO (4.0 mg, 0.025 mmol), and this mixture was stirred for a few minutes to dissolve the reagents. Then, the corresponding laccase was added (12 U/mL) and the reaction was shaken in an orbital shaker at 250 rpm and 30 ºC for 2.5 h. The reaction mixture was acidified using HCl (3 M) and extracted with DCM (2 x 5 mL). The combined organic layers were washed with brine and dried over Na_2SO_4. The residue was analysed by GC and NMR (Table S1). Control reaction without the laccase showed no detectable conversion.

Table S1. Laccase screening for the oxidation of 2-phenyl-1-propanol (**1**).



rac-**1** **2** **3**

Entry	Laccase	c (%)	**1** (%)	**2** (%)	**3** (%)
1	*Trametes versicolor*	95	5	68	27
2	*Trametes* sp.	12	88	9	3
3	*M. thermopila*	<1	>99	<1	<1
4	*Cerrena unicolor*	53	45	33	20
5	*Pleurotus ostreatus*	<1	>99	<1	<1

Optimising parameters

Our previously reported conditions for the oxidation of **1** using *Trametes versicolor*/TEMPO as catalytic system are shown in Table S2 (entry 1).[2] In this work, the reaction was performed under more concentrated conditions and monitored within the time (entries 2-4, Table S2). Using these conditions, the pH and concentration of substrate for the subsequent bioreduction could be fixed easily by dilution or adjustment of the pH by the addition of a base. The general protocol is given below:

In a 25 mL erlenmeyer flask, *rac*-alcohol **1** (150 mg, 1.13 mmol) was dissolved in citrate buffer (10 mL; 50 mM, pH 5.5). Then, TEMPO (30 mg, 0.19 mmol) and laccase from *Trametes versicolor* (55 mg) were added. The reaction mixture was shaken at 30 °C and 250 rpm in an orbital shaker open to air for 3.5 h. The reaction mixture was

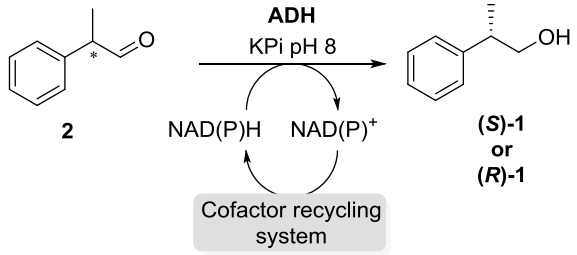
acidified using HCl (3 M) and extracted with DCM (2 x 5 mL). The residue was analysed by GC (Table S2).

Table S2. Laccase/TEMPO-mediated oxidation of **1** at different substrate concentrations.

Entry	t (h)	Conc (mM)	Conv (%)[a]
1	2	30	85 (15)
2	2	90	60
3	3.5	90	>97 (7)
4	5	90	>97 (35)

[a] Conversion values were determined by GC analysis. The percentage of carboxylic acid is indicated in brackets.

III. Reduction of racemic 2-phenylpropionaldehyde (2) with ADHs



Method A (used for all ADHs except RasADH): In a 1.5 mL vial, 2-phenylpropionaldehyde **2** (3 mg, 0.022 mmol) was dissolved in phosphate buffer (700 µL, 50 mM, pH 8) containing 1 mM NAD(P)H and the cosubstrate (EtOH or 2-PrOH, 5% v v^{-1}). $MgCl_2$ was added for LBADH and Evo-1.1.200 (1 mM). Finally, the ADH (3 U) was added. Reactions were shaken at 30ºC and 250 rpm for 22 h and extracted with EtOAc (2 x 0.5 mL). The organic layer was separated by centrifugation (2 min, 13000 rpm) and dried over Na_2SO_4. Conversions and enantiomeric excess were determined by GC and HPLC respectively (see Table 1 in Manuscript).

Method B (used for RasADH): In a 1.5 mL vial, 2-phenylpropionaldehyde **2** (3 mg, 0.022 mmol) was dissolved in phosphate buffer (700 µL, 50 mM, pH 8) containing 1 mM NADPH, glucose (100 mM) and GDH (20 U). Then, *E. coli*/RasADH (10 mg) was added. The reaction was shaken at 30ºC and 250 rpm for 22 h and stopped by extraction with EtOAc (2 x 0.5 mL). The organic layers were combined and dried over Na_2SO_4.

The solvent was evaporated and conversions were determined by GC and *ee* by chiral HPLC (see Table 1 in Manuscript).

Optimising parameters

In order to improve the enantioselectivities for the preparation of alcohol **1**, additional experiments were performed changing parameters such as pH and temperature. The general protocol is described below:

In a 1.5 mL vial, 2-phenylpropionaldehyde **2** (3 mg, 0.022 mmol) was dissolved in phosphate buffer at different pHs as indicated in Table S3 (700 μL, 50 mM) containing 1 mM NAD(P)H and the cosubstrate (EtOH or 2-PrOH, 5% v v^{-1}). $MgCl_2$ was added for Evo-1.1.200 (1 mM). Finally, the ADH was added. Reactions were shaken at the temperature indicated in Table S3 and 250 rpm for 22 h and extracted with EtOAc (2 x 0.5 mL). The organic layer was separated by centrifugation (2 min, 13000 rpm) and dried over Na_2SO_4. Conversions and enantiomeric excess were determined by GC and HPLC, respectively.

Note: Under more drastic conditions, the *ee* slightly increases in some cases but more acetophenone was detected.[3]

Table S3. Bioreductions of **2** at different reaction conditions.

Entry	pH, T	ADH	c (%)[a]	*ee* (%)[b]
1	pH 8, 30 °C	ADH-P	>97 (10)	94 (*S*)
2	pH 8, 60 °C	ADH-P	>97 (14)	88 (*S*)
3	pH 9, 30 °C	ADH-P	95	86 (*S*)
4	pH 8, 30 °C	HLADH	>97 (5)	90 (*S*)
5	pH 8, 60 °C	HLADH	>97 (16)	94 (*S*)
6	pH 9, 30 °C	HLADH	>97 (14)	88 (*S*)
7	pH 10, 30 °C	HLADH	>97 (14)	94 (*S*)
8	pH 9, 30 °C	Evo-1.1.200	>97 (5)	90 (*R*)
9	pH 9, 60 °C	Evo-1.1.200	>97 (10)	94 (*R*)
10	pH 10, 30 °C	Evo-1.1.200	97 (6)	88 (*R*)
11	pH 10, 45 °C	Evo-1.1.200	90 (10)	>97 (*R*)

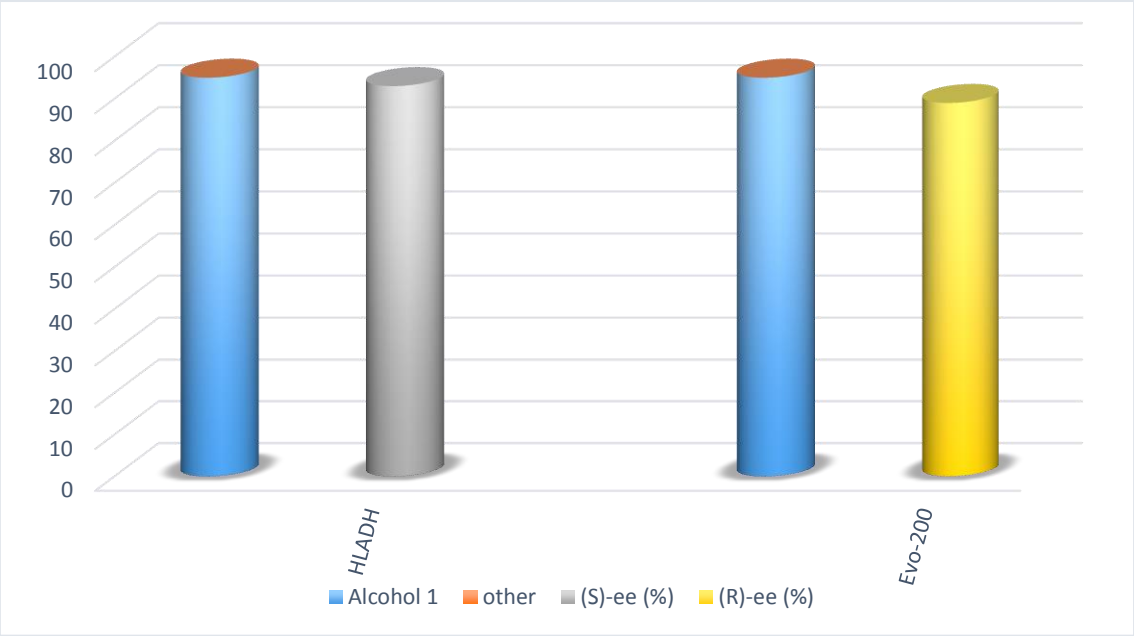
[a] Conversions were determined by GC and the amount of acetophenone is indicated in brackets. [b] Enantiomeric excess were determined by chiral HPLC.

IV. Bioreduction of 2-phenylpropionaldehyde (2) at 500 mg-scale

In a 25 mL erlenmeyer flask, *rac*-**2** (0.5 g, 3.6 mmol) was dissolved in phosphate buffer (115 mL, pH 8 for HLADH and pH 9 for Evo-1.1.200) containing 5% v/v of cosubstrate (EtOH for HLADH and 2-PrOH for Evo-1.1.200) and 1 mM of NADH. In the case of Evo-1.1.200, 1 mM of $MgCl_2$ was added. Finally, the ADH (10 mg) was added to the reaction mixture and after 3 h, additional 10 mg were added. The reaction was monitored by GC and stopped when no aldehyde was remaining (7 h).

Evo-1.1.200, conv= 95%, *ee*= 89% (*R*).

HLADH, conv= 93%, *ee*= 93% (*S*).



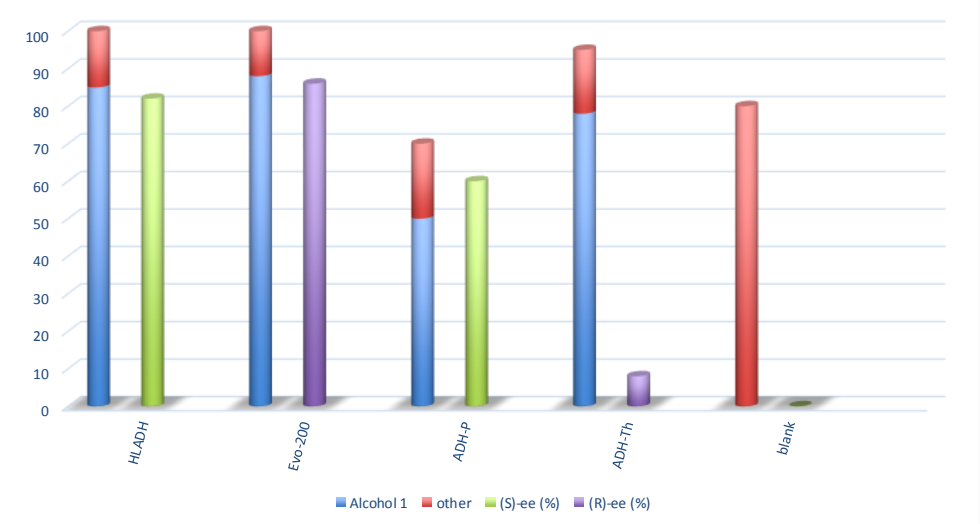
V. Two-step two-pot deracemisation biotransformation

In a 25 mL erlenmeyer flask, *rac*-alcohol **1** (150 mg, 1.13 mmol) was dissolved in citrate buffer (10 mL; 50 mM citrate, pH 5.5). TEMPO (30 mg, 0.19 mmol) and laccase from *Trametes versicolor* (55 mg) were added. The reaction mixture was shaken at 30 °C and 250 rpm for 3.5 h. After that time, the reaction mixture was extracted with EtOAc (2 × 500 µL) and dried over Na_2SO_4. The conversion was determined by achiral GC. Then the residue crude was redissolved in phosphate buffer (30 mL, 50 mM, pH 8 or 9) containing 5% v/v of cosubstrate (EtOH or 2-PrOH) and 1 mM NAD(P)H. Finally, the ADH (20 mg for overexpressed HLADH, 50 mg for overexpressed ADH-P and 3 mg for Evo-1.1.200) was added. Reactions were shaken at 30ºC and 250 rpm for 24 h and extracted with DCM (3 x 30 mL). The organic layer was separated by centrifugation (2 min, 13000 rpm) and dried over Na_2SO_4. Conversions and enantiomeric excess were determined by GC and HPLC, respectively.

VI. Two-step one-pot deracemisation reaction

In a 25 mL erlenmeyer flask, *rac*-alcohol **1** (150 mg, 1.13 mmol) was dissolved in citrate buffer (10 mL; 50 mM, pH 5.5). Then, TEMPO (30 mg, 0.19 mmol) and laccase from *Trametes versicolor* (55 mg) were added. The reaction mixture was shaken at 30 °C and 250 rpm for 3.5 h. After that time, the reaction mixture was diluted by adding phosphate buffer (30 mL) and the pH was carefully adjusted (pH 8 or 9) containing 5%

v/v of EtOH or 2-PrOH and 1 mM NAD(P)H. Finally, the ADH (20 mg for overexpressed HLADH and 3 mg for Evo-1.1.200) was added. The reaction was shaken for 24 h at 30 ºC and 250 rpm. After that time, the reaction was extracted with DCM (3 × 30 mL) and the organic extract dried over Na_2SO_4. The conversion was determined by achiral GC and the *ee* by HPLC. Acetophenone was detected as byproduct (in red).[3]



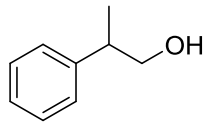
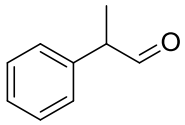
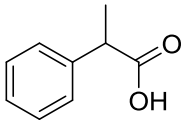
HLADH, conversion >97%, 82% (*S*) *ee*
132 mg crude 88 %
Isolated yield: 62%

Evo-1.1.200, conversion 93%, 86% (*R*) *ee*
160 mg crude
Isolated yield: 71%

ADH-P, conversion 70%, 60% (*S*) *ee*
Slower reaction and big amount of acetophenone (20%) by NMR and GC.

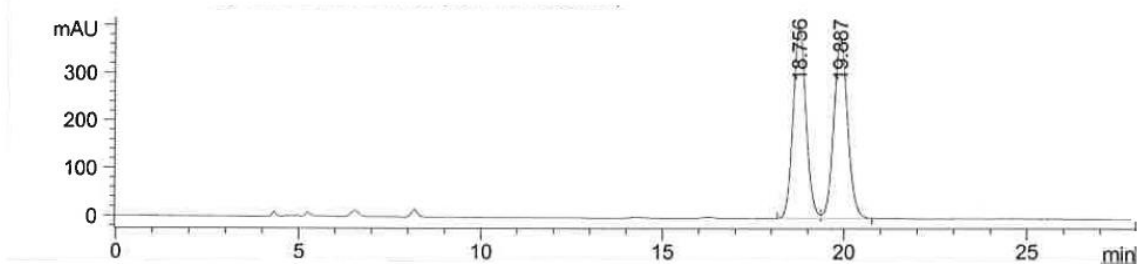
Blank experiments
Without ADHs or with *E. coli*:
77-82% of acetophenone formed in both cases by NMR.

VII. Analytical methods

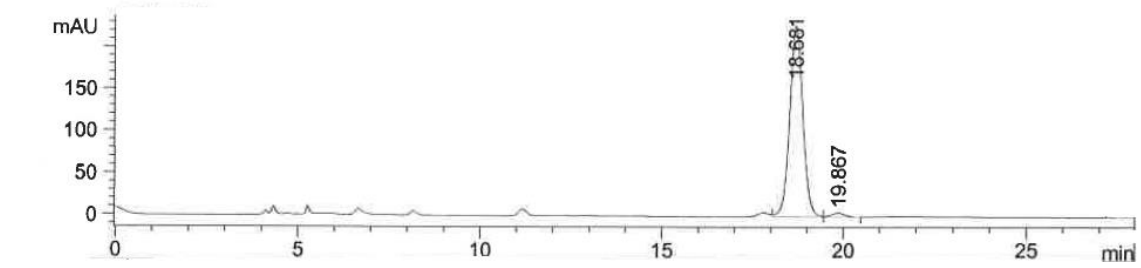
Compound	GC[a]		Chiral HPLC or GC[b]	
	Method	t_R (min)	Method	t_R (min)
	90/3/10/220/2	4.1	**A**	18.7, 19.7
	90/3/10/220/2	3.5	**B**	18.1, 18.4
	90/3/10/220/2	6.0	-	-

[a] Hewlett Packard HP-1 column (30 m x 0.32 mm x 0.25 µm, 15 psi) was used to determine the conversion value. The program is as follows: initial temp. (°C)/ time (min)/ slope (°C/min)/ final temp. (°C)/ time (min). [b] Separations for determining the *ee* were performed in **A**: Daicel IA column (25cm x 4.6 mm I.D) at 210 nm using 98:2 hexane/2-PrOH as eluent and flow 0.7 mL/min at 40 ºC or **B**: Restek RT-BetaDEXse column (30 m x 0.25 mm x 0.25 µm, 12.2 psi N_2), program: 70/0/3/180/0.

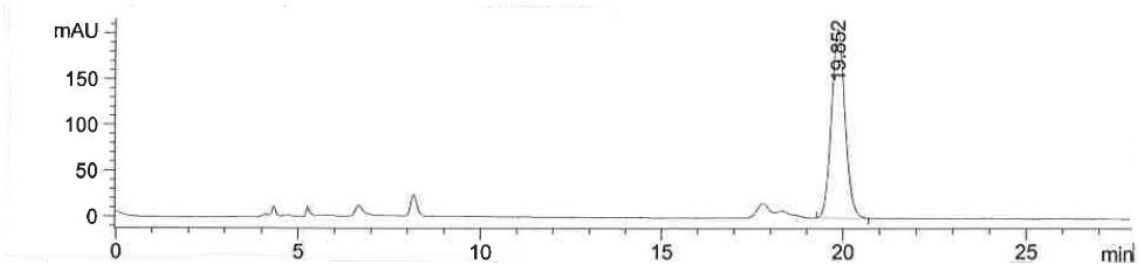
rac-**1**



(*S*)-**1** HLADH reaction



(*R*)-**1** Evo-1.1.200 reaction



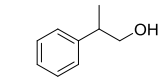
VIII. References

1. a) I. Lavandera, A. Kern, V. Resch, B. Ferreira-Silva, A. Glieder, W. M. F. Fabian, S. de Wildeman and W. Kroutil, *Org. Lett.*, 2008, **10**, 2155-2158; b) I. Lavandera, A. Kern, B. Ferreira-Silva, A. Glieder, S. de Wildeman and W. Kroutil, *J. Org. Chem.*, 2008, **73**, 6003-6005.

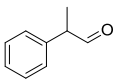
2. A. Díaz-Rodríguez, L. Martínez-Montero, I. Lavandera, V. Gotor and V. Gotor-Fernández, *Adv. Synth. Catal.*, 2014, **356**, 2321-2329.

3. a) D. P. Riley, D. P. Getman, G. R. Beck and R. M. Heintz, *J. Org. Chem*., 1987, **52**, 287-290; b) C. S. Fuchs, M. Hollauf, M. Meissner, R. C. Simon, T. Besset, J. N. H. Reek, W. Riethorst, F. Zepeck and W. Kroutil, *Adv. Synth. Catal.*, 2014, **356**, 2257-2265.

IX. NMR spectra

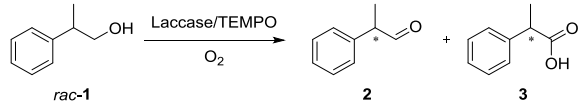


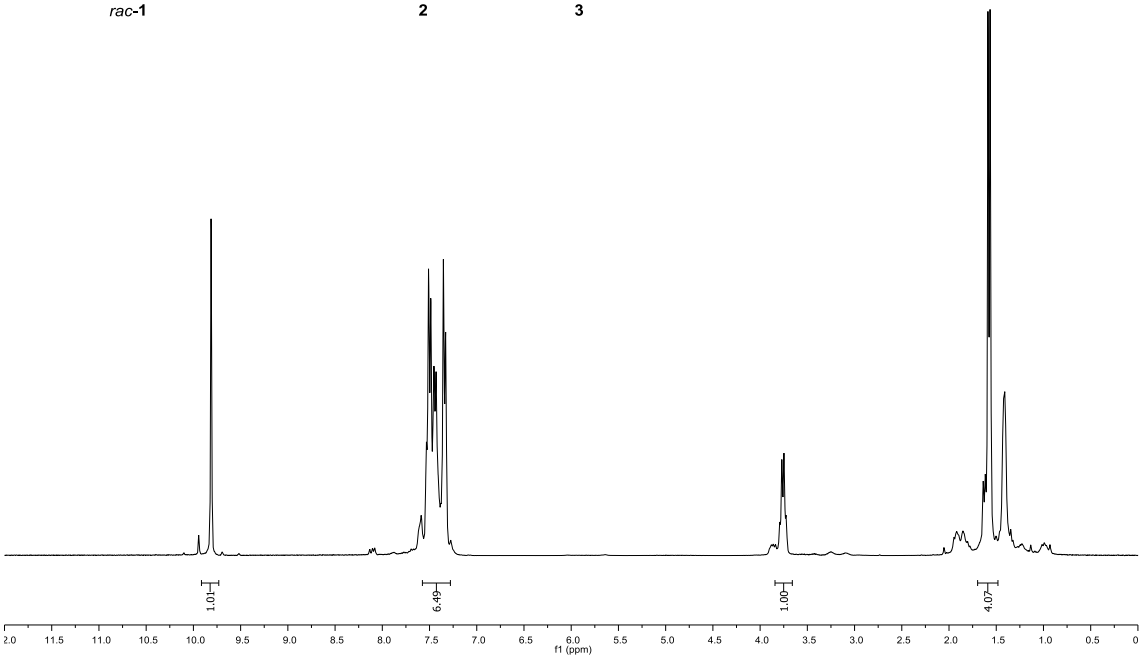
rac-**1**



rac-**2**

Two-step two-pot transformation (crude material after laccase/TEMPO-mediated oxidation)





Two-step one-pot reaction (crude material after bioreduction with HLADH)