

Supplementary data for article:

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Supporting Information

Highly efficient Michael-type addition of acetaldehyde to β -nitrostyrenes by whole resting cells of *Escherichia coli* expressing 4-oxalocrotonate tautomerase

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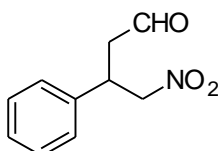
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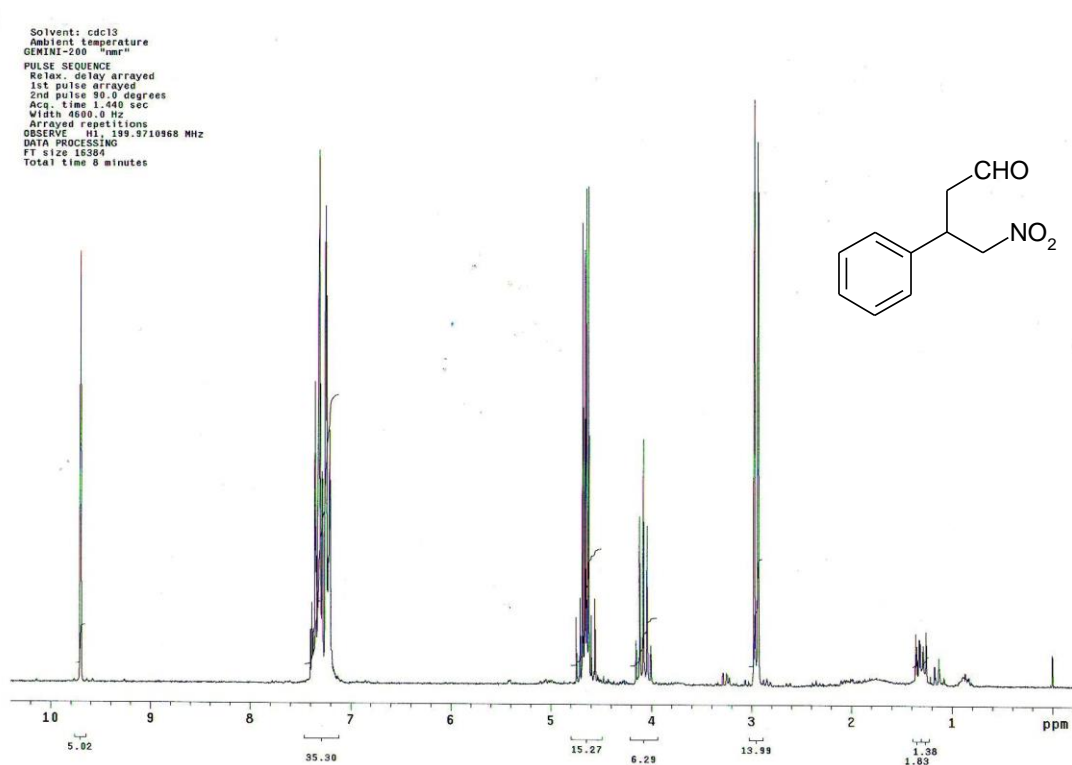
S1(A-D) Characterization data and NMR spectra of addition products obtained by biotransformation using *E. coli* BL21(4-OT)

^1H and ^{13}C NMR spectra were recorded on a Varian Gemini 200 at 200/50 MHz in deuterated chloroform (CDCl_3). The chemical shifts were expressed as δ values in ppm using tetramethylsilane as internal standard and the coupling constants (J) are in Hertz (Hz). The following abbreviations were used for signal multiplicities: s, singlet; d, doublet; qu, quintet; and m, multiplet. NMR data of known compounds are in agreement with literature values.

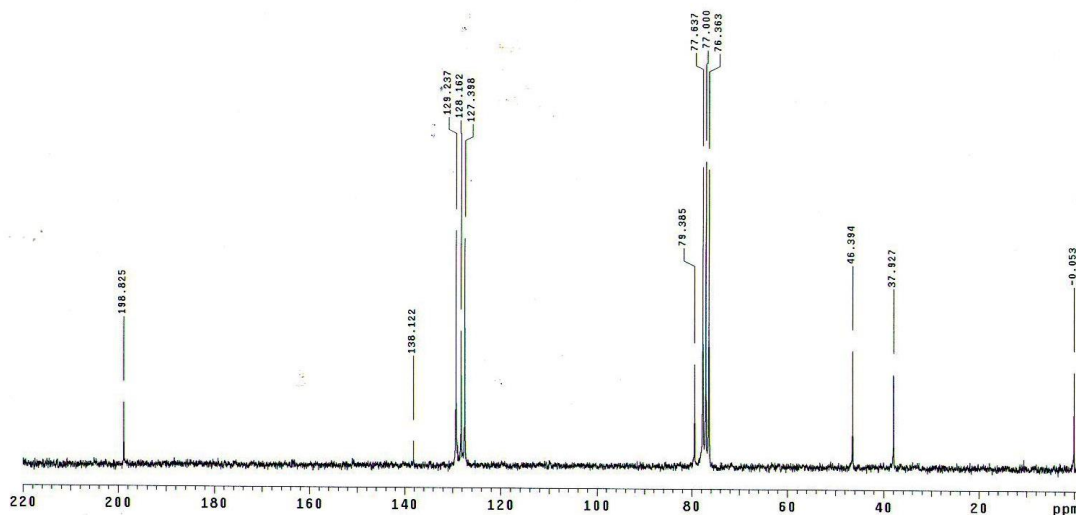
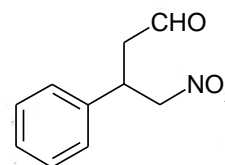
A) 4-nitro-3-phenylbutanal (3)¹



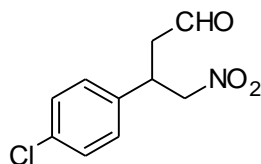
^1H NMR (200 MHz): 9.71 (s, 1H); 7.40-7.20 (m, 5H); 4.74-4.56 (m, 2H); 4.08 (qu, 1H, $J_1=7.3$ Hz, $J_2=7.3$ Hz); 2.94 (d, 2H, $J=6.8$ Hz). ^{13}C NMR (50 MHz): 198.8; 138.1; 129.2; 128.2; 127.4; 79.4; 46.4; 37.9.



JRVM038
 Solvent: cdc13
 Ambient temperature
 GEMINI-200 "nm"
 PULSE SEQUENCE
 Relax, delay arrayed
 1st pulse arrayed
 2nd pulse 81.8 degrees
 Acq. time 1.067 sec
 Width 15900.0 Hz
 Arrayed repetitions
 OBSERVE C13, 50.2827782 MHz
 DECOUPLE H1, 199.9712607 MHz
 Power 0 dB
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 WALTZ-16 modulated
 DATA PROCESSING
 Line broadening 1.5 Hz
 FT size 32768
 Total time 14.9 hours

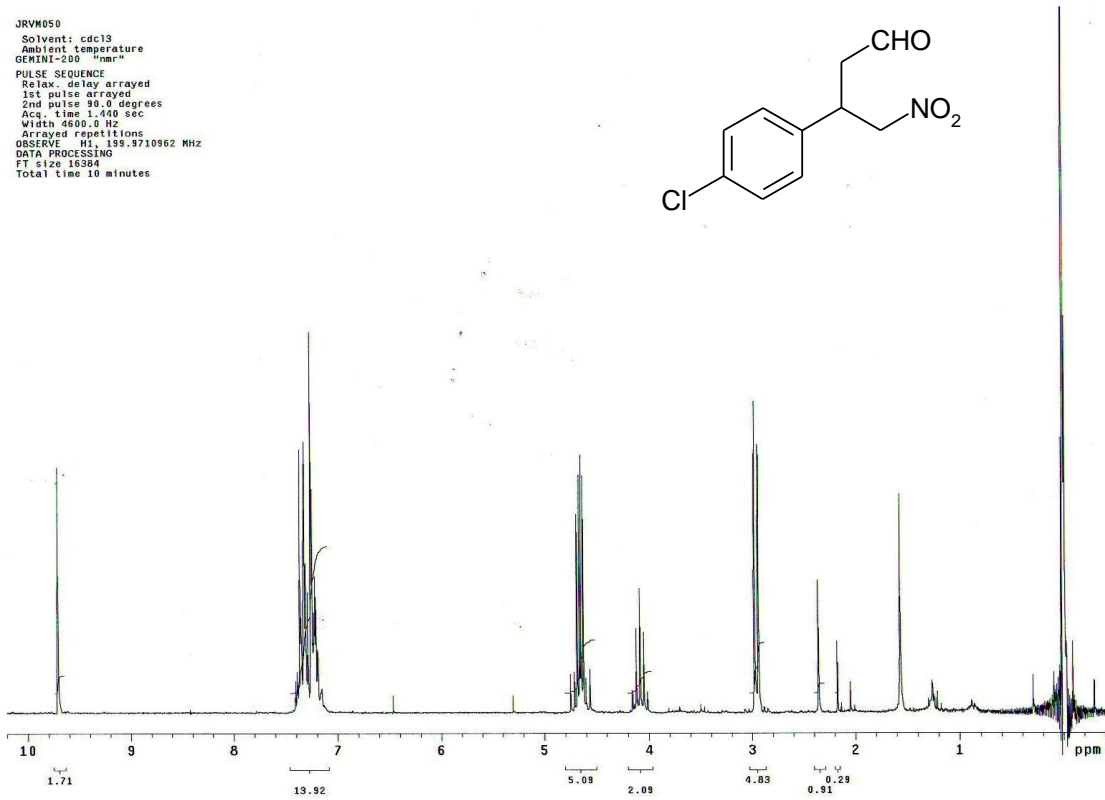
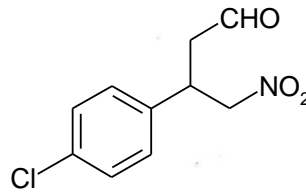


B) 3-(4-chlorophenyl)-4-nitrobutanal (5)

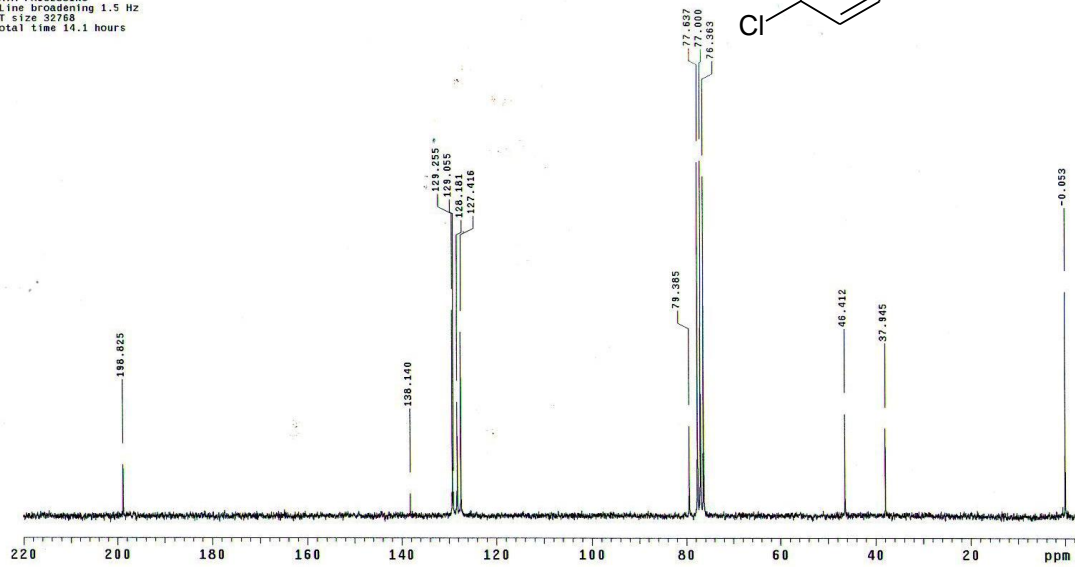
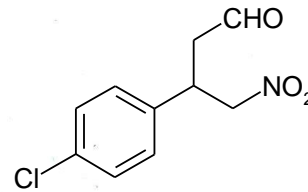


^1H NMR (200 MHz): 9.71 (s, 1H); 7.40-7.15 (m, 4H); 4.74-4.56 (m, 2H); 4.08 (qu, 1H, $J_1=7.3$ Hz, $J_2=7.3$ Hz); 2.95 (d, 2H, $J=6.6$ Hz). ^{13}C NMR (50 MHz): 198.8; 138.1; 129.3; 128.2; 127.4; 79.4; 46.4; 37.9.

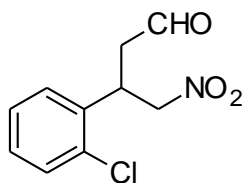
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 FT size 16384
 Total time 10 minutes



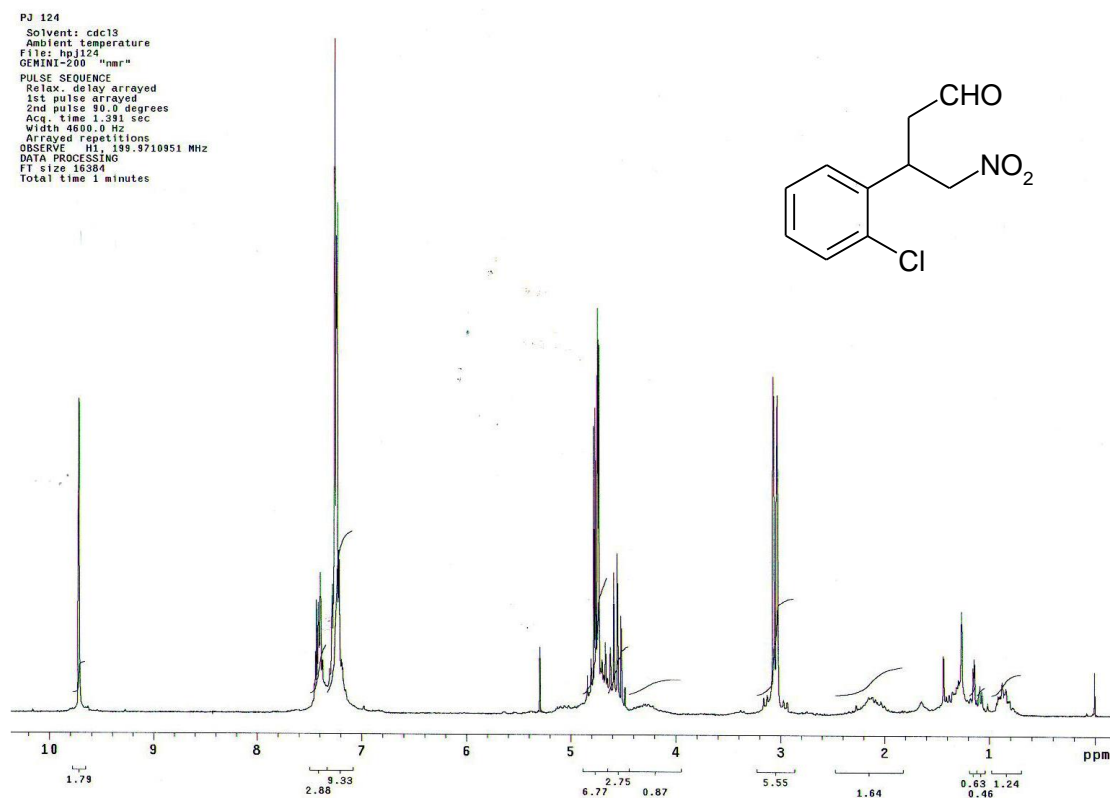
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 Ambient temperature
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 Arrayed repetitions
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 DATA PROCESSING
 Line broadening 1.5 Hz
 FT size 32768
 Total time 14.1 hours



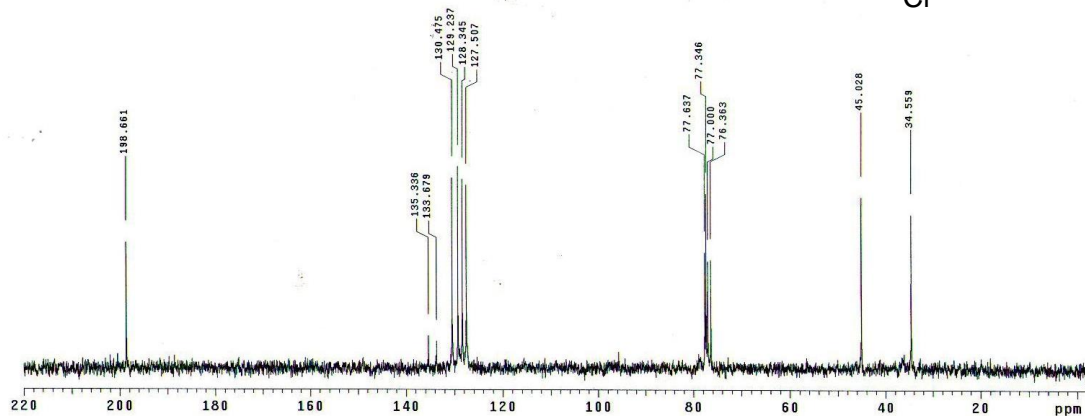
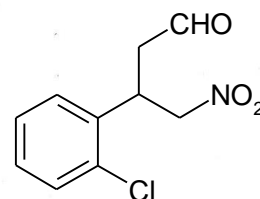
C) 3-(2-chlorophenyl)-4-nitrobutanal (7)¹



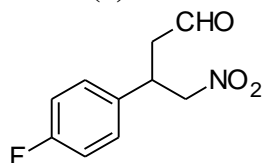
¹H NMR (200 MHz): 9.72 (s, 1H); 7.45-7.18 (m, 4H); 4.83-4.48 (m, 3H); 3.05 (d, 2H, *J*=7.4Hz). ¹³C NMR (50MHz): 198.7; 135.3; 133.7; 130.5; 129.2; 128.4; 127.5; 77.3; 45.0; 34.5.



PJ-124
 Solvent: cdcl3
 Ambient temperature
 File: cp124
 GEMINI-200 "nmr"
 PULSE SEQUENCE
 Relax. delay arrayed
 1st pulse arrayed
 2nd pulse 81.8 degrees
 Acq. time 1.987 sec
 Width 15000.0 Hz
 Arrayed repetitions
 OBSERVE C13, 50.2827800 MHz
 DECOUPLE H1, 199.8712807 MHz
 Power 0 dB
 continuously on
 WALTZ-16 modulated
 DATA PROCESSING
 Line broadening 1.5 Hz
 FT size 32768
 Total time 23 minutes

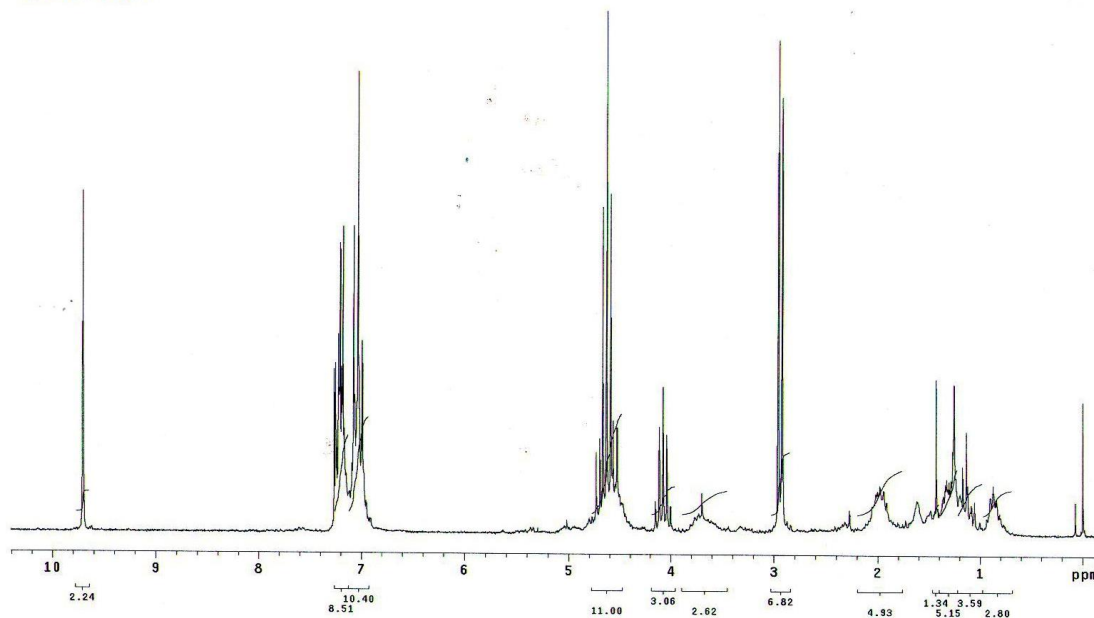
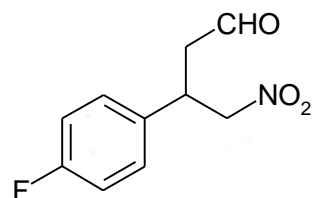


D) 3-(4-fluorophenyl)-4-nitrobutanal (9)¹

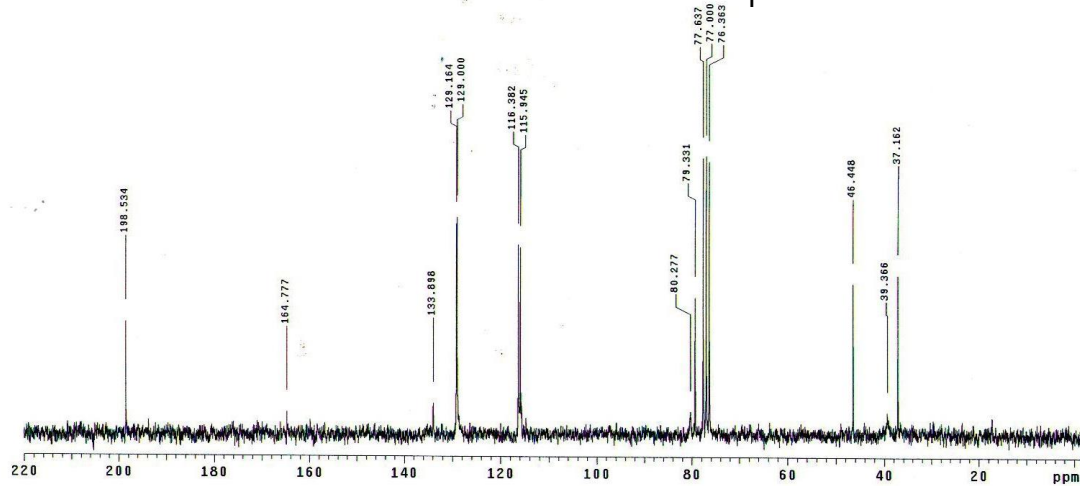
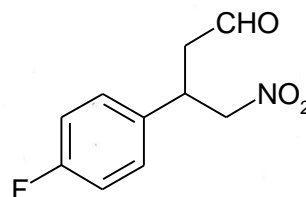


¹H NMR (200 MHz): 9.71(s, 1H); 7.26-6.95 (m, 4H); 4.72-4.45 (m, 2H); 4.00 (qu, 1H, $J_1=7.3$ Hz, $J_2=7.3$ Hz); 2.94 (d, 2H, $J=7.2$ Hz). ¹³C NMR (50 MHz): 198.5; 133.9; 129.2; 129.0; 115.9; 79.3; 46.4; 37.2.

PJ 121-1
 Solvent: cdc13
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 2nd pulse 90.0 degrees
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 Width 4600.0 Hz
 Arrayed repetitions
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 DATA PROCESSING
 FT size 16384
 Total time 2 minutes



PJ 121-1
 Solvent: cdc13
 Ambient temperature
 GEMINI-200 "nmr"
 PULSE SEQUENCE
 Relax. delay arrayed
 1st pulse arrayed
 2nd pulse 81.8 degrees
 Acq. time 1.067 sec
 Width 15000.0 Hz
 Arrayed repetitions
 OBSERVE C13, 50.2827782 MHz
 DECOUPLE H1, 199.9712807 MHz
 Power 0 dB
 continuously on
 WALTZ-16 modulated
 DATA PROCESSING
 Line broadening 1.5 Hz
 FT size 32768
 Total time 104 minutes



S2 (A-B) Synthesis of 4-amino-3-phenylbutanol (10) from biotransformation product 3

A) 4-nitro-3-phenylbutanol ²

To a stirred solution of 4-nitro-3-phenylbutanal (3) (0.1 mmol) in anhydrous methanol (2.0 ml) NaBH₄ (0.15 mmol) was added at 0°C. The reaction was stirred for 30 min and quenched with H₂O and extracted with ethyl acetate (3 times 10 ml). Combined organic layers were washed with brine and dried (MgSO₄), filtered and the solvent was removed under reduced pressure. The obtained crude product was purified by flash column chromatography on silica gel (ethyl acetate/hexanes 1:1).

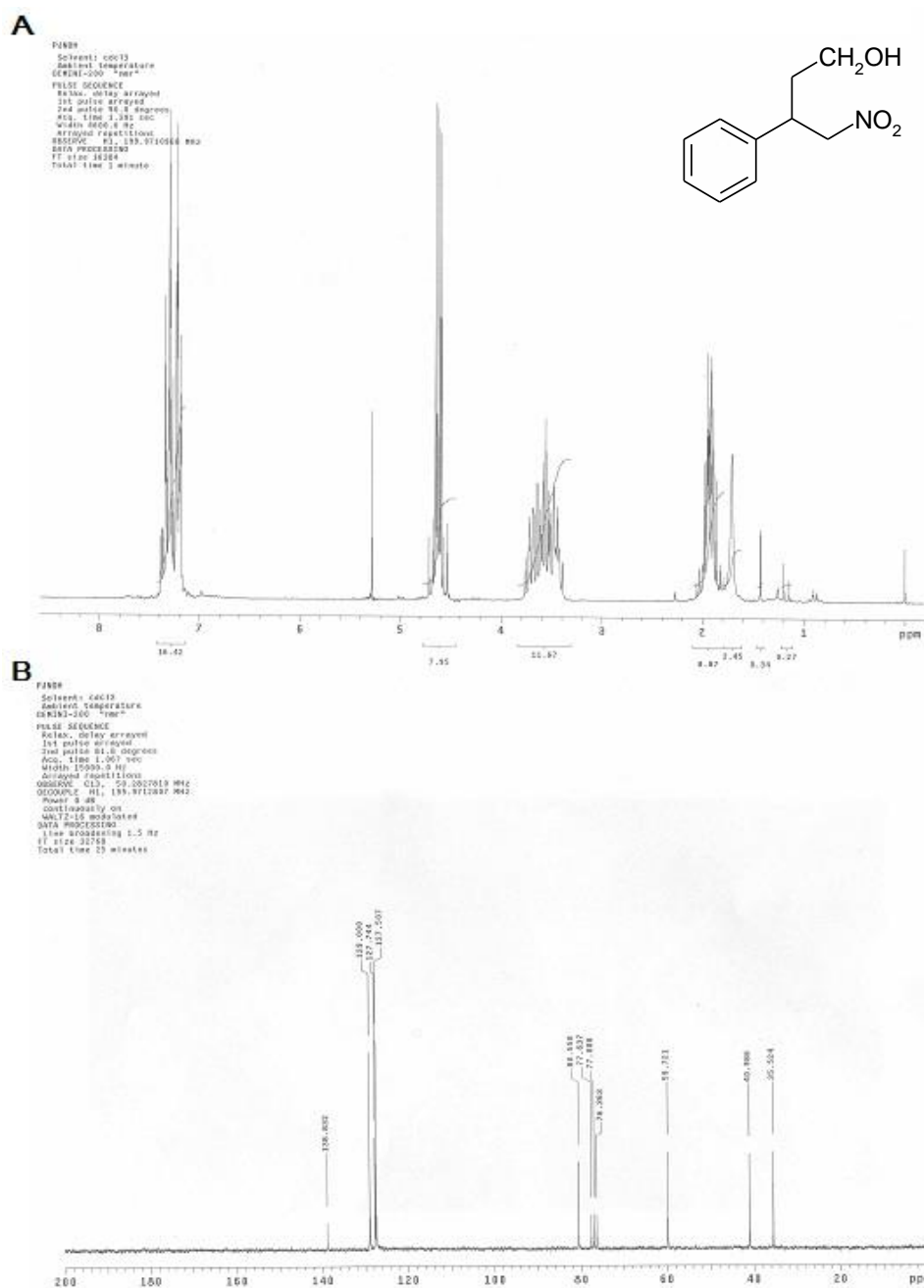


Fig. S2-A. A) ¹H NMR spectrum of prepared 4-nitro-3-phenylbutanol (200 MHz): 7.38-7.18 (m, 5H); 4.71-4.53 (m, 2H); 3.76-3.40 (m, 3H); 2.05-1.80 (m, 2H), 1.71 (s, 1H). B) ¹³C NMR (50 MHz): 138.8; 129.0; 127.7; 127.5; 80.5; 59.7; 41.0; 35.5.

B) 4-amino-3-phenylbutanol (10) ³

A mixture of 4-nitro-3-phenylbutanol (20 mg, 0.1 mmol) and 10% Pd(OH)₂ on carbon (10 mg) in anhydrous methanol (20 ml) was hydrogenated at 60 psi for 4 h using Parr apparatus. The solution was filtered and concentrated to give 4-amino-3-phenylbutanol as viscous oil (17 mg, 99%).

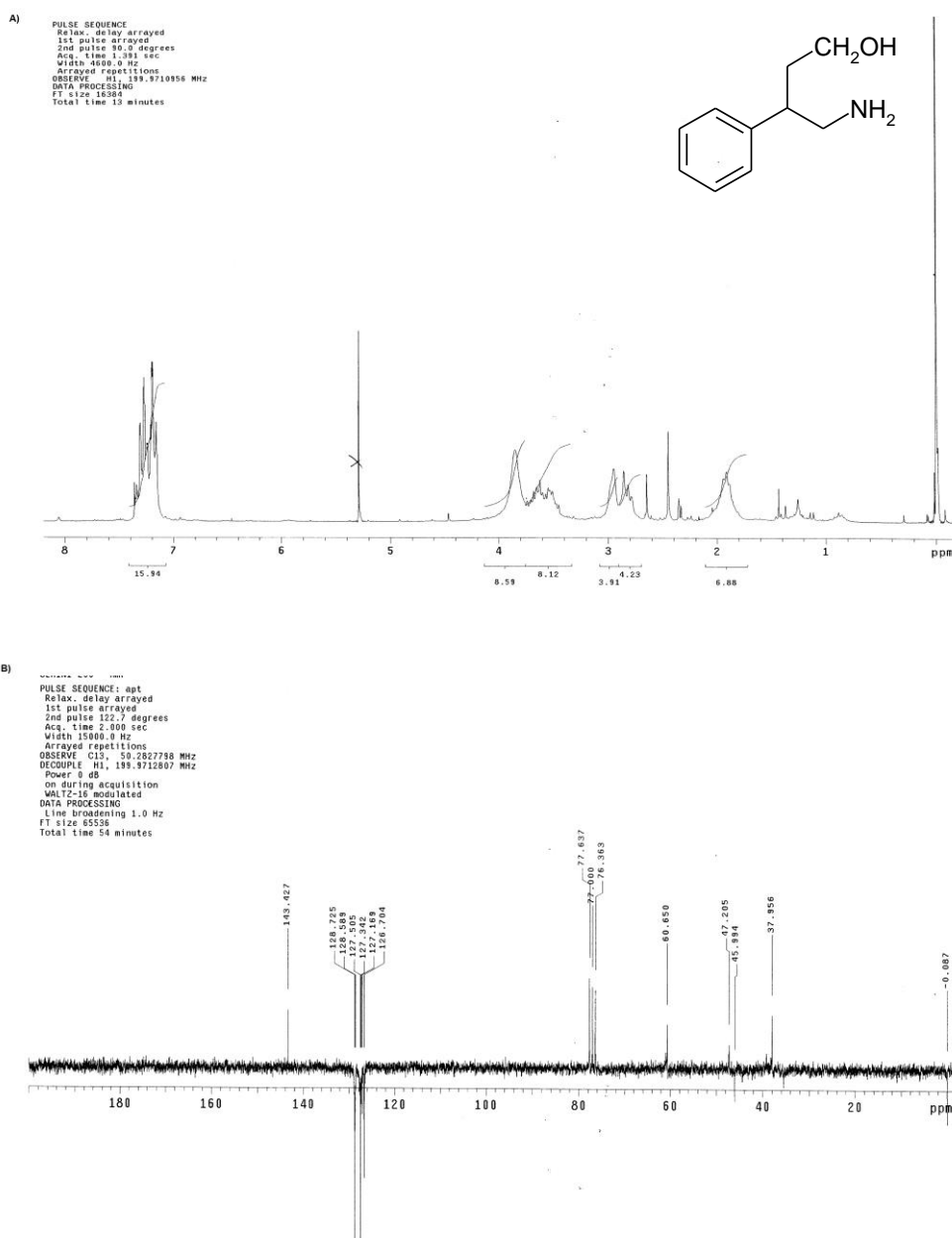


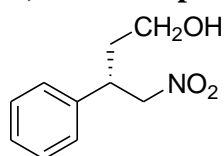
Fig. S2-B. A) ¹H NMR spectrum of prepared 4-amino-3-phenylbutanol (10). B) ¹³C NMR (50 MHz): 143.4; 128.7; 127.5; 126.7; 60.7; 47.2; 46.0; 38.0.

S3(A-D) Chiral analysis of biotransformation products

All biotransformation products (3, 5, 7 and 9) were firstly reduced to alcohols by the procedure described under S2-A due to the instability upon storage at -20°C.

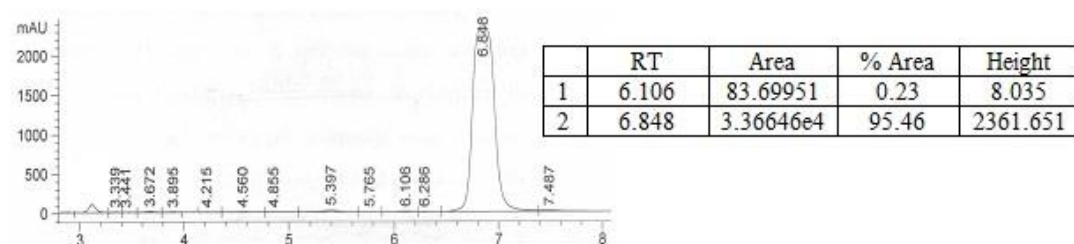
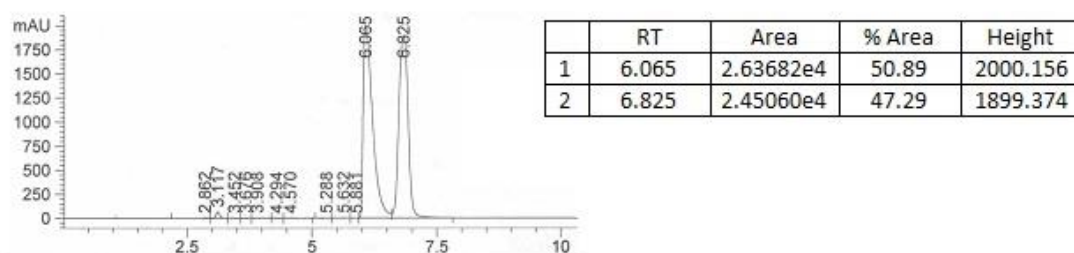
Racemic mixture of the samples was prepared chemically from the corresponding aldehydes using the same procedure. The enantiomeric excess was determined by HPLC (Agilent Technologies, HP110) with CHIRALPAK IA column (Chiral Technologies Europe, Cedex, France) at 210 nm for all samples.

A) 4-nitro-3-phenylbutan-1-ol (obtained from biotransformation product 3)

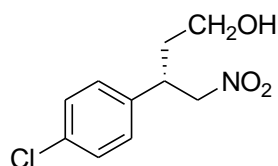


$[\alpha]_D^{25} = -22.0$ (c 1.0, CH₂Cl₂, >99% ee).

HPLC conditions: heptane/iPrOH in the ratio of 80/20, flow rate = 1.0 ml/min (tr=6.06 min, tr=6.83 major), ee >99%.

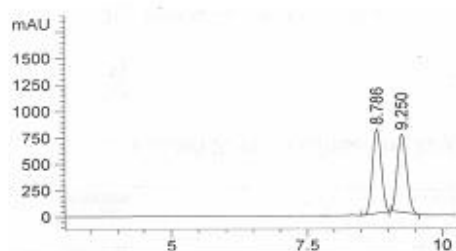


B) 3-(4-chlorophenyl)-4-nitrobutan-1-ol (obtained from biotransformation product 5) ⁴

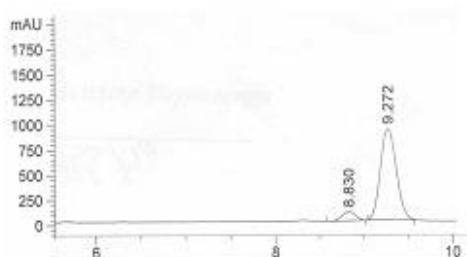


$[\alpha]_D^{25} = -11.5$ (c 2.0, CH₂Cl₂, 84% ee). ¹H NMR (200 MHz): 7.35-7.27 (m, 2H); 7.19-7.14 (m, 2H); 4.71-4.52 (m, 2H); 3.77-3.56 (m, 2H); 3.51-3.39 (m, 1H);

2.06-1.79 (m, 2H); 1.68 (s, 1H). ^{13}C NMR (50 MHz): 137.4; 133.5; 129.2; 128.9; 80.3; 59.5; 40.4; 35.4. HPLC conditions: heptane/ethanol in the ratio of 80/20, flow rate = 1.0 ml/min (tr=8.83 min, tr=9.27 major), ee 84%.

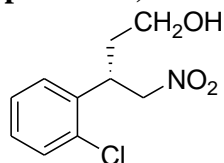


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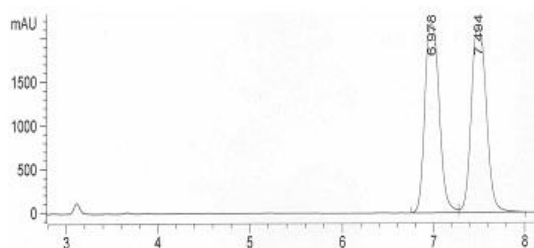


	RT	Area	% Area	Height
1	8.830	948.6715	7.761	89.732
2	9.272	1.12738e4	92.238	905.526

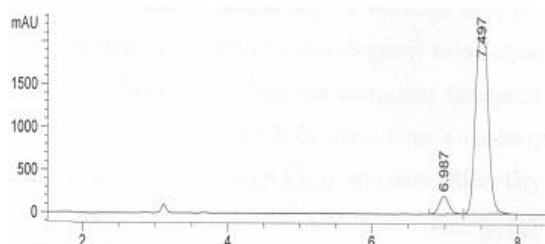
C) 3-(2-chlorophenyl)-4-nitrobutan-1-ol (obtained from biotransformation product 7)



$[\alpha]_{\text{D}}^{25} = +4$ (c 1.0, CH_2Cl_2 , 88% ee). ^1H NMR (200 MHz): 7.44-7.39 (m, 1H); 7.28-7.18 (m, 3H); 4.81-4.64 (m, 2H); 4.36-4.21 (m, 1H); 3.69-3.49 (m, 2H); 2.08-1.98 (m, 2H); 1.60 (s, 1H). ^{13}C NMR (50 MHz): 136.3; 134.2; 130.4; 128.9; 128.2; 127.5; 78.9; 59.9; 37.3; 34.7. HPLC conditions: heptane/*i*PrOH in the ratio of 80/20, flow rate = 1.0 ml/min (tr=6.98 min, tr=7.50 major), ee 88%.

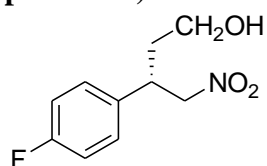


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1	6.978	2.38763e4	49.237	2168.684
2	7.494	2.46157e4	50.762	2130.952



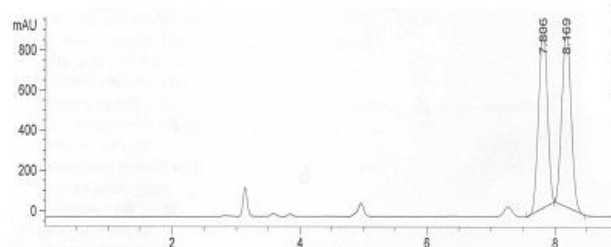
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1	6.987	1989.6008	6.964	213.228
2	7.497	2.65793e4	93.036	2205.303

D) 3-(4-fluorophenyl)-4-nitrobutan-1-ol (obtained from biotransformation product 9)

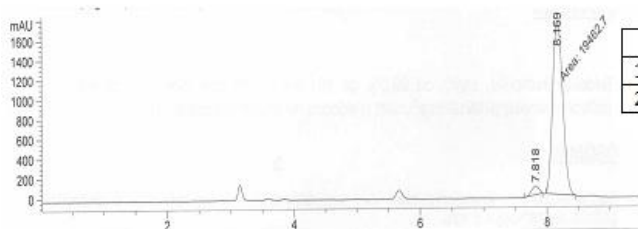


$[\alpha]_D^{25} = -10$ (c 1.0, CH_2Cl_2 , 94% ee). ^1H NMR (200 MHz): 7.27-7.16 (m, 2H); 7.09-6.98 (m, 2H); 4.72-4.52 (m, 2H); 3.80-3.59 (m, 2H); 3.54-3.42 (m, 1H); 2.07-1.78 (m, 2H); 1.58 (s, 1H). ^{13}C NMR (50 MHz): 137.9; 134.5; 129.2; 129.1; 80.6; 59.7; 40.3; 35.6.

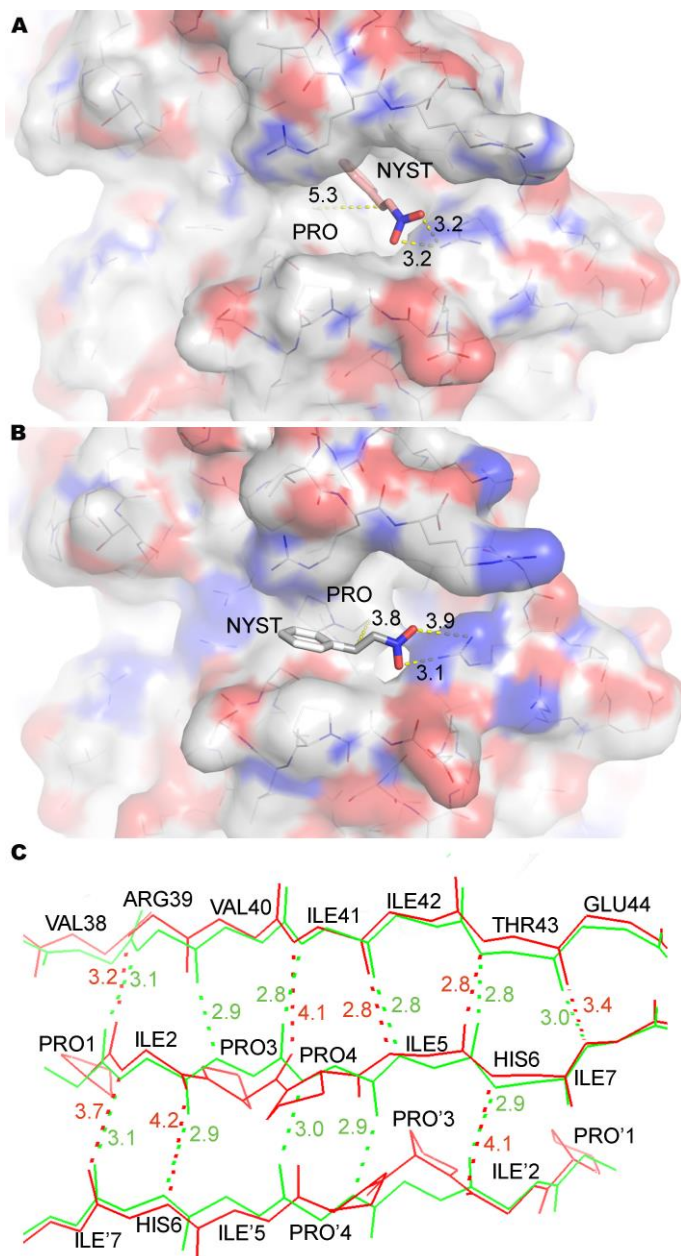
HPLC conditions: heptane/ethanol in the ratio of 80/20, flow rate = 1.0 ml/min (tr=7.81 min, tr=8.17 major), ee 94%.



	RT	Area	% Area	Height
1	7.806	1048.9473	49.557	110.99449
2	8.170	1067.6752	50.442	106.31933



	RT	Area	% Area	Height
1	7.818	692.28418	3.434	85.04726
2	8.169	1.94627e4	96.565	1751.9398



S4. A) Nitrostyrene (NYST) in stick representation positioned in the reactive center of wild type tautomerase (line and surface representation). B) Nitrostyrene (NYST) positioned in the reactive center of 4-OT_P mutant (line and surface representation). Distances to ARG'11 residue and acetaldehyde bonded to the terminal PRO are given. C) Backbone representation of the wild type (green) and 4-OT_2P variant (red) tautomerase with the first β -plate in the center. Only Proline residues are shown with the side chains and only the variant residues are labelled. Distances between hydrogen bond forming peptide bond oxygen and nitrogen atoms together with their distances are provided.

References

1. Wang, Y.; Li, P.; Liang, X.; Zhang, T. Y.; Ye, J., An efficient enantioselective method for asymmetric Michael addition of nitroalkanes to α,β -unsaturated aldehydes. *Chem. Commun.* **2008**, *2008*, 1232-1234.
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4. Qiao, Y.; He, J.; Ni, B.; Headley, A. D., Asymmetric Michael reaction of acetaldehyde with nitroolefins catalyzed by highly water-compatible organocatalysts in aqueous media. *Adv. Synth. Catal.* **2012**, *354*, 2849-2853.