

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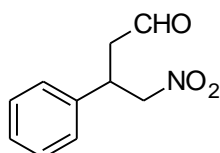
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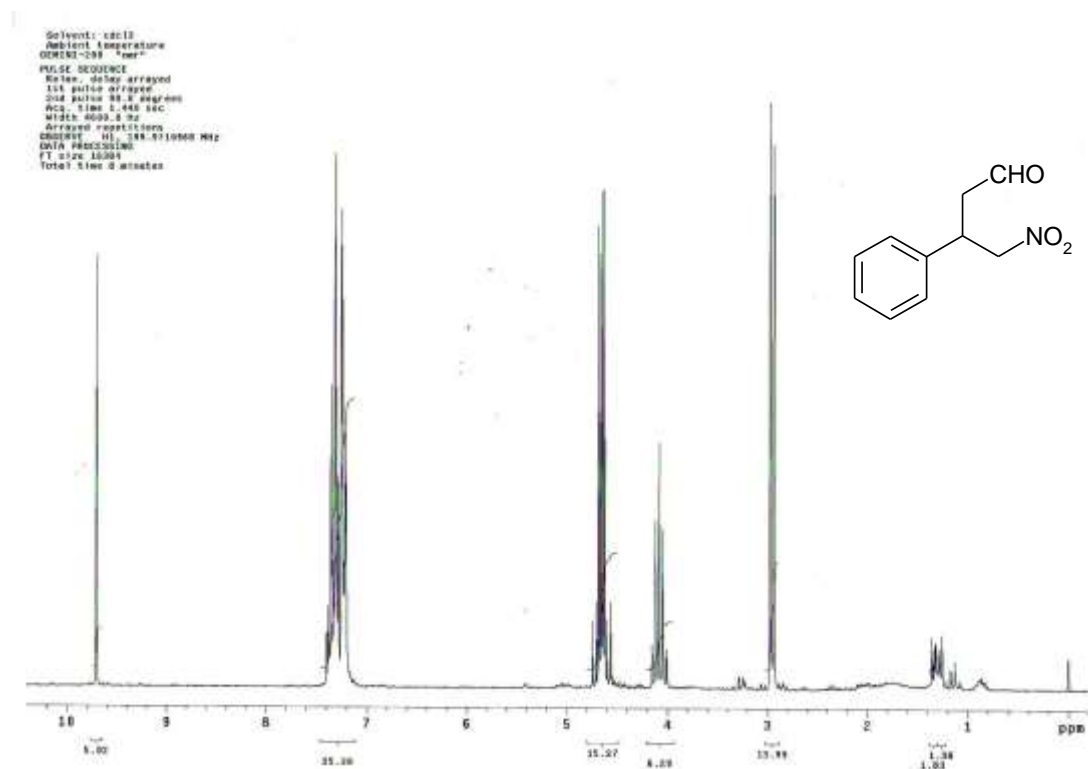
S1(A-D) Characterization data and NMR spectra of addition products obtained by biotransformation using *E.coli* BL21(4-OT)

^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



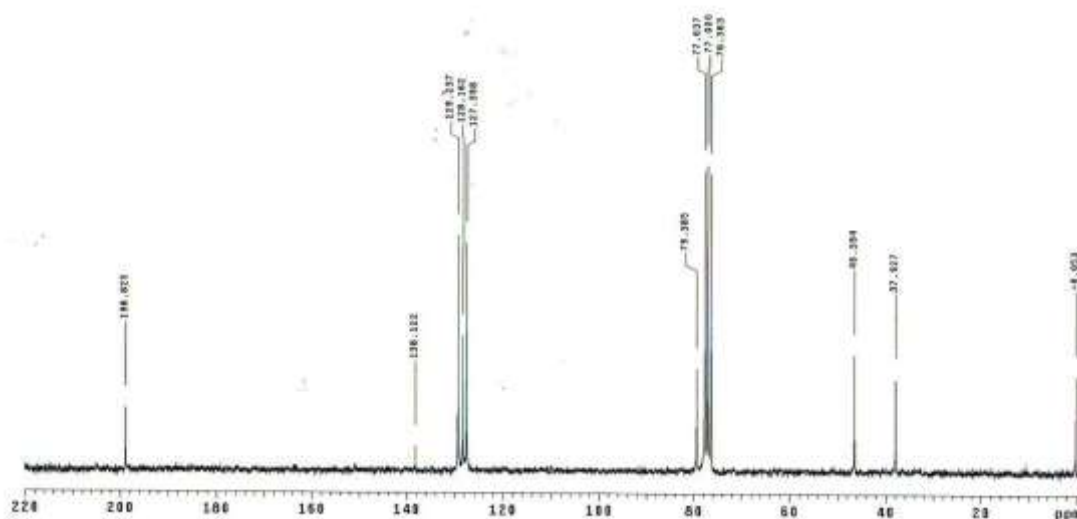
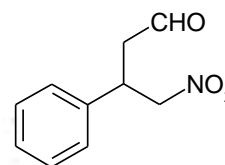
^1H NMR (200 MHz): 9.71 (s, 1H); 7.40-7.20 (m, 5H); 4.74-4.56 (m, 2H); 4.08 (qu, 1H, $J_1=7.3$ Hz, $J_2=7.3$ Hz); 2.94 (d, 2H, $J=6.8$ Hz). ^{13}C NMR (50 MHz): 198.8; 138.1; 129.2; 128.2; 127.4; 79.4; 46.4; 37.9.



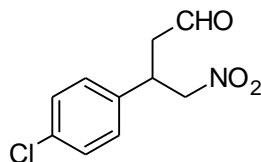
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Acquire repetitions
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DATA PROCESSING
Line broadening 1.5 Hz
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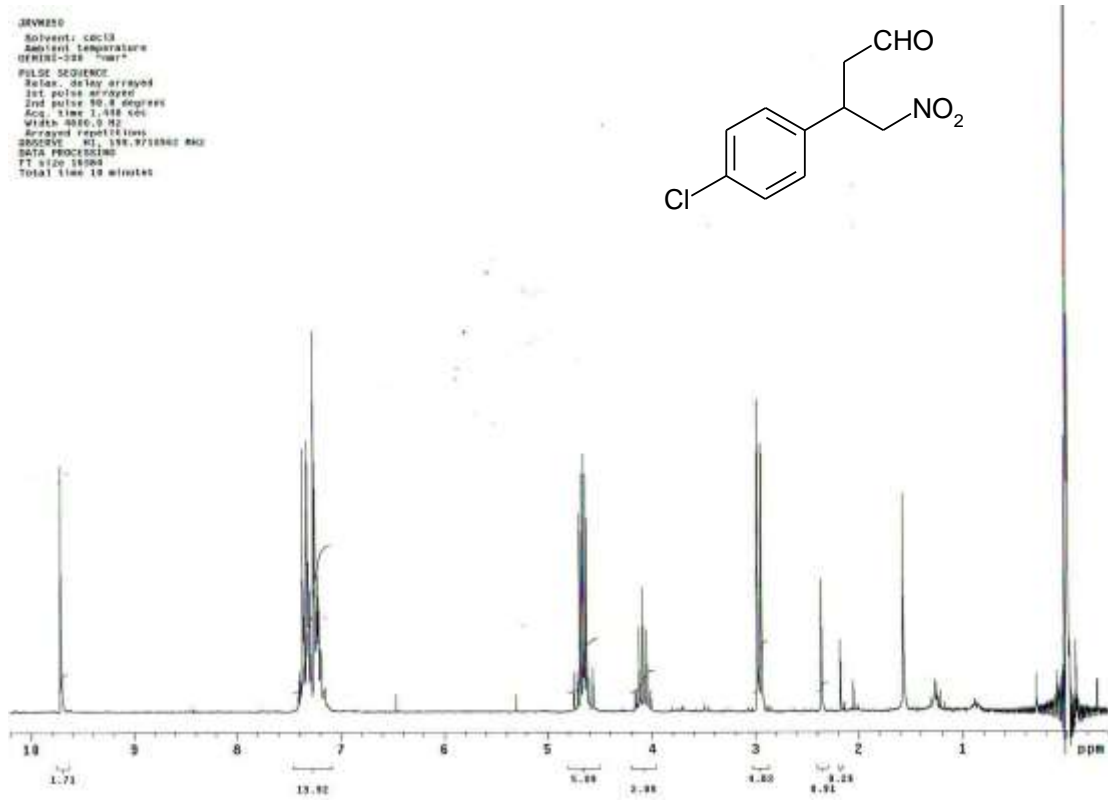
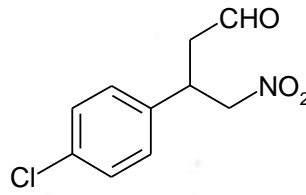


B) 3-(4-chlorophenyl)-4-nitrobutanal (5)

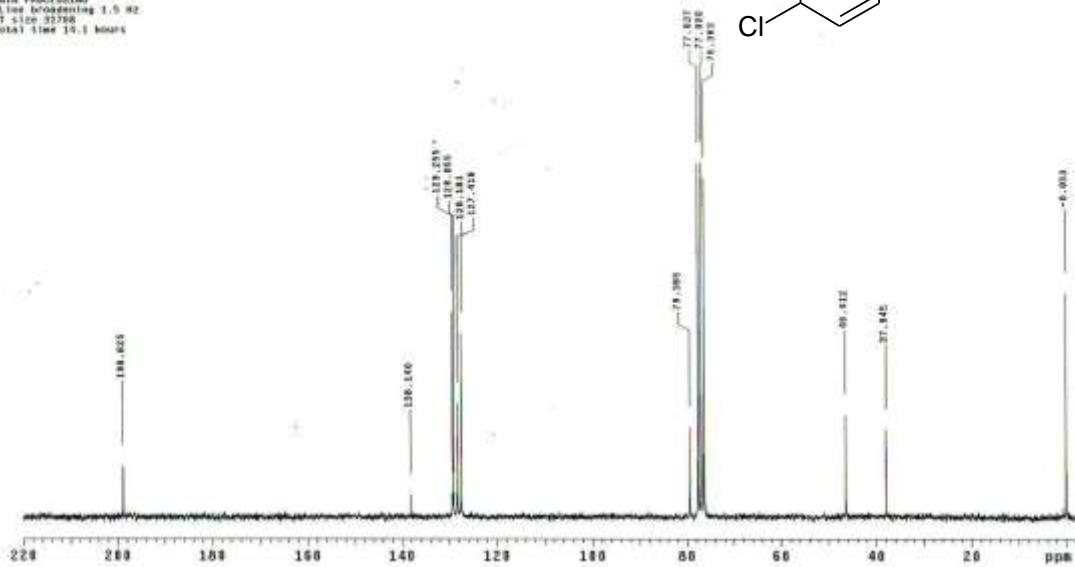
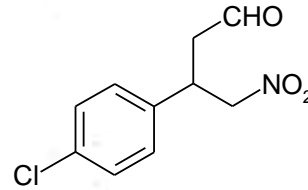


^1H NMR (200 MHz): 9.71 (s, 1H); 7.40-7.15 (m, 4H); 4.74-4.56 (m, 2H); 4.08 (qu, 1H, $J_1=7.3$ Hz, $J_2=7.3$ Hz); 2.95 (d, 2H, $J=6.6$ Hz). ^{13}C NMR (50 MHz): 198.8; 138.1; 129.3; 128.2; 127.4; 79.4; 46.4; 37.9.

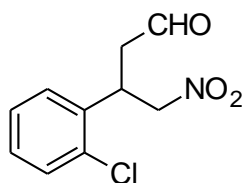
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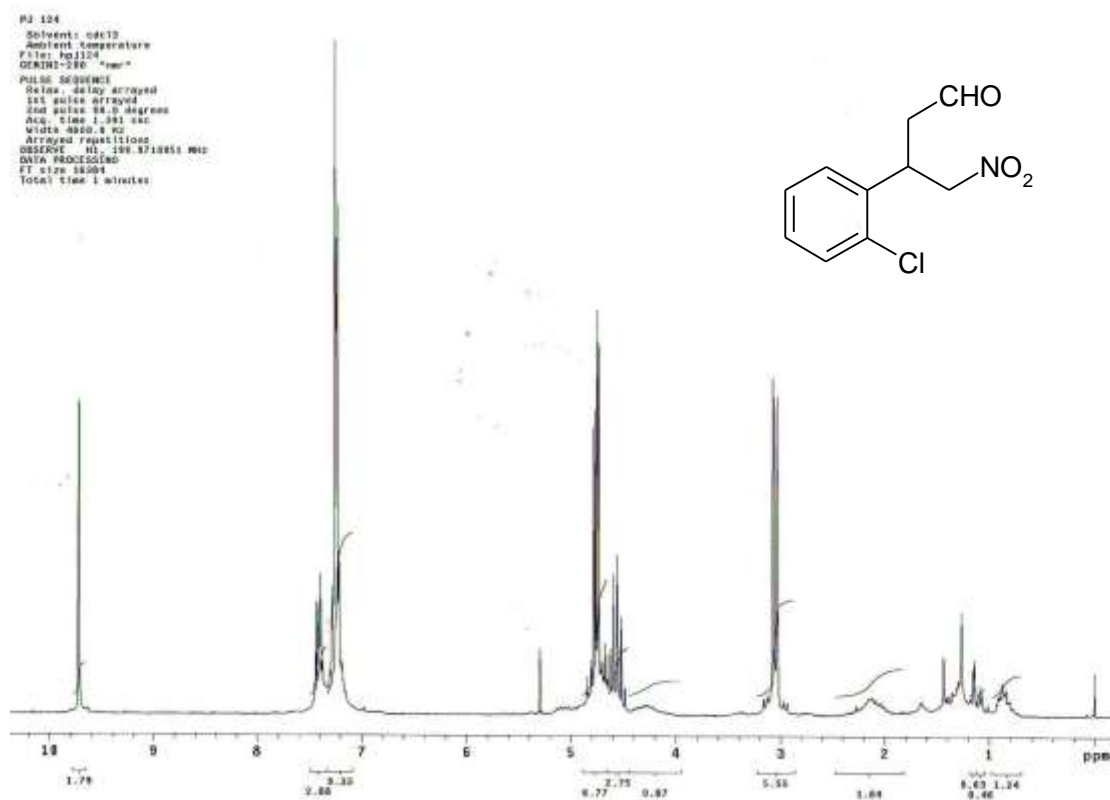
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 Scs. time 1.000 sec
 Width 10000.0 Hz
 Arrayed repetitions
 2500000 Hz, 198.3710000 MHz
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 Power 0 dB
 Constantly on
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 DATA PROCESSING
 Line broadening 1.5 Hz
 FT size 32768
 Total time 19.1 hours



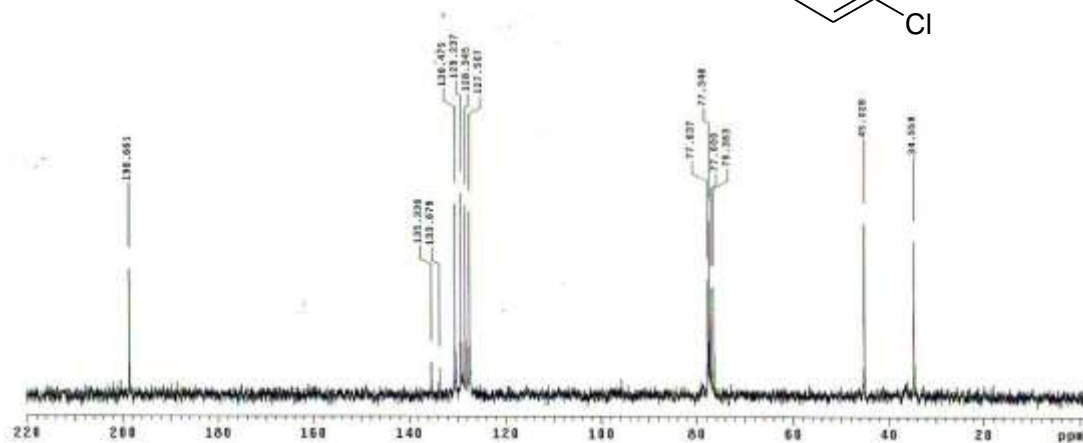
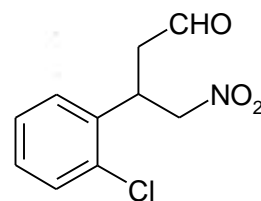
C) 3-(2-chlorophenyl)-4-nitrobutanal (7)¹



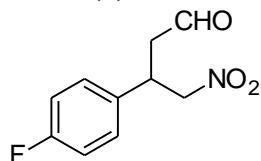
¹H NMR (200 MHz): 9.72 (s, 1H); 7.45-7.18 (m, 4H); 4.83-4.48 (m, 3H); 3.05 (d, 2H, *J*=7.4Hz). ¹³C NMR (50MHz): 198.7; 135.3; 133.7; 130.5; 129.2; 128.4; 127.5; 77.3; 45.0; 34.5.



FD-124
 Solvent: d613
 Solvent Temperature:
 Filter: 0.124
 GEMINI-200 "var"
 PULSE SEQUENCE
 Relax, delay arrayed
 1st pulse arrayed
 2nd pulse 91.8 degree
 Acq. Time 1.887 sec
 Width 15000.0 Hz
 Arrayed repetitions
 SFOURV CL1, 50.2827860 MHz
 DC000PL1 H1, 100.6210007 MHz
 Power 8.00
 Continuously on
 WA12-20 modulated
 DATA MODULATED
 Line Broadening 1.0 Hz
 FT C13a 32748
 Total Time 28 minutes

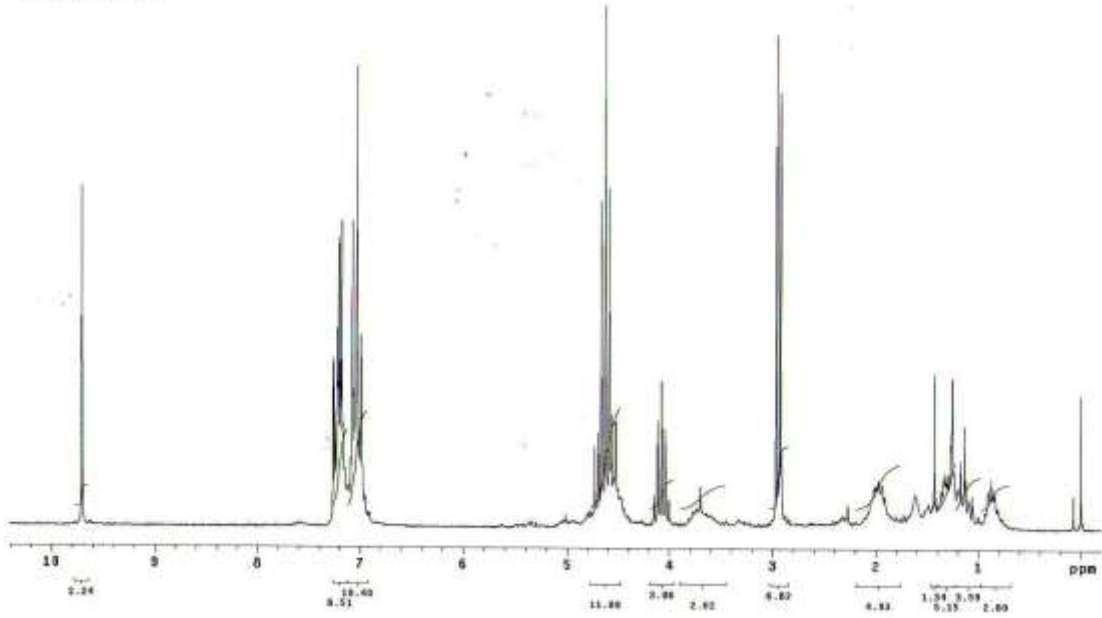
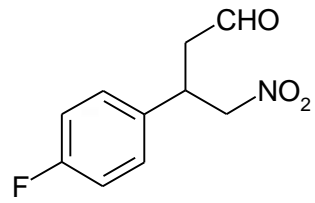


D) 3-(4-fluorophenyl)-4-nitrobutanal (9)¹

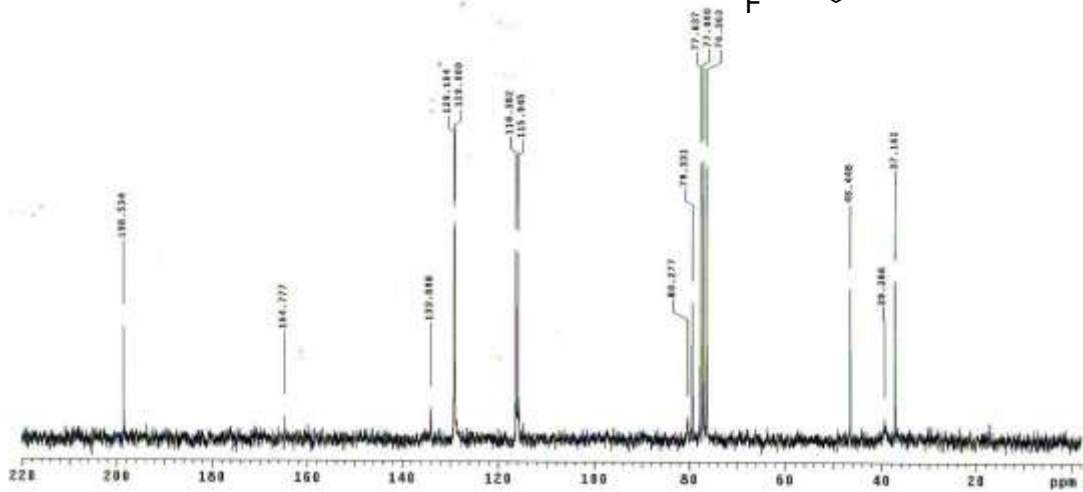
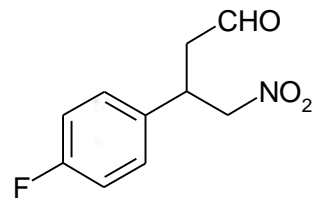


¹H NMR (200 MHz): 9.71(s, 1H); 7.26-6.95 (m, 4H); 4.72-4.45 (m, 2H); 4.00 (qu, 1H, $J_1=7.3$ Hz, $J_2=7.3$ Hz); 2.94 (d, 2H, $J=7.2$ Hz). ¹³C NMR (50 MHz): 198.5; 133.9; 129.2; 129.0; 115.9; 79.3; 46.4; 37.2.

PJ 121-1
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 1st pulse arrayed
 2nd pulse 90.0 degrees
 Acq. time 1.007 sec
 Width 8000.0 Hz
 Arrayed positions
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 DATA PROCESSING
 FT size 16384
 Total time 2 minutes



PJ 121-1
 Solvent: cdcl3
 Ambient temperature
 QNP1H-200 "nmr"
 PULSE SEQUENCE
 Relax delay arrayed
 1st pulse arrayed
 2nd pulse 91.0 degrees
 Acq. time 1.007 sec
 Width 15000.0 Hz
 Arrayed positions
 OBSERVE F1, 50.2627902 MHz
 DECOUPLE H1, 100.5712807 MHz
 Power: 0 dB
 continuity on
 MWTZ-16 module
 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).

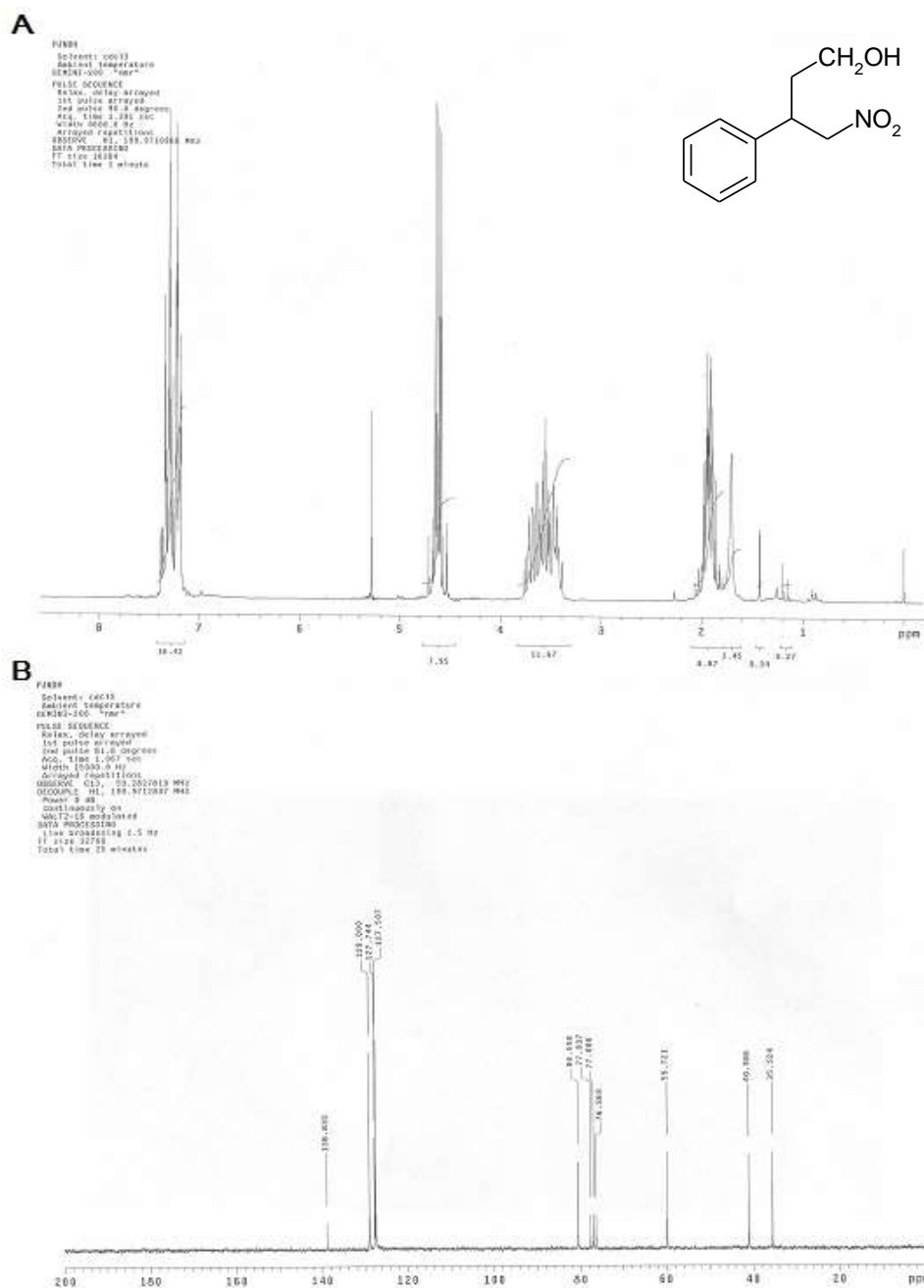


Fig. S2-A. A) ¹H NMR spectrum of prepared 4-nitro-3-phenylbutanol (200 MHz): 7.38-7.18 (m, 5H); 4.71-4.53 (m, 2H); 3.76-3.40 (m, 3H); 2.05-1.80 (m, 2H), 1.71 (s, 1H). B) ¹³C NMR (50 MHz): 138.8; 129.0; 127.7; 127.5; 80.5; 59.7; 41.0; 35.5.

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%).

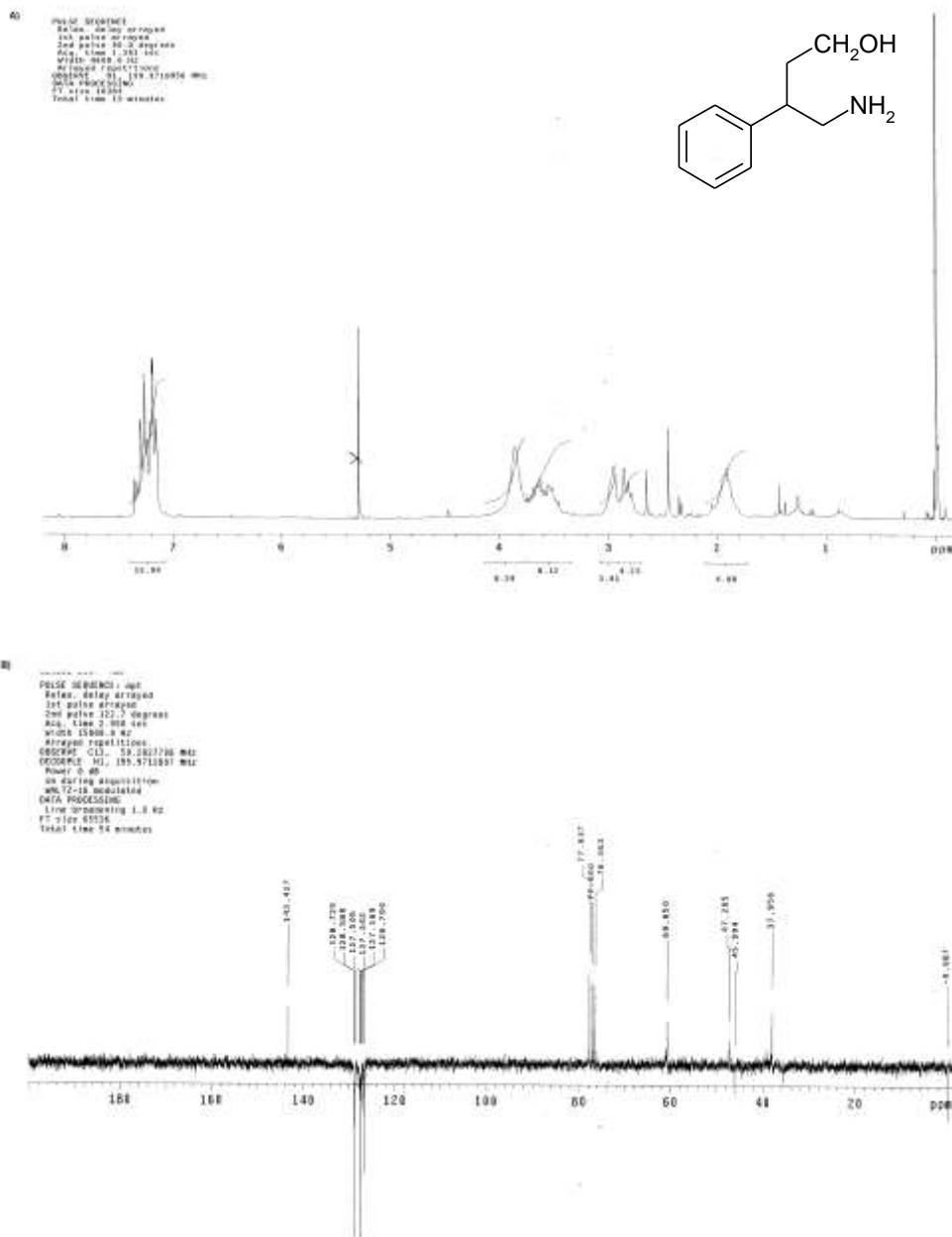


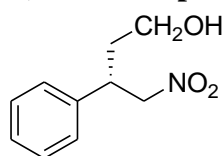
Fig. S2-B. A) ¹H NMR spectrum of prepared 4-amino-3-phenylbutanol (**10**). B) ¹³C NMR (50 MHz): 143.4; 128.7; 127.5; 126.7; 60.7; 47.2; 46.0; 38.0.

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 .

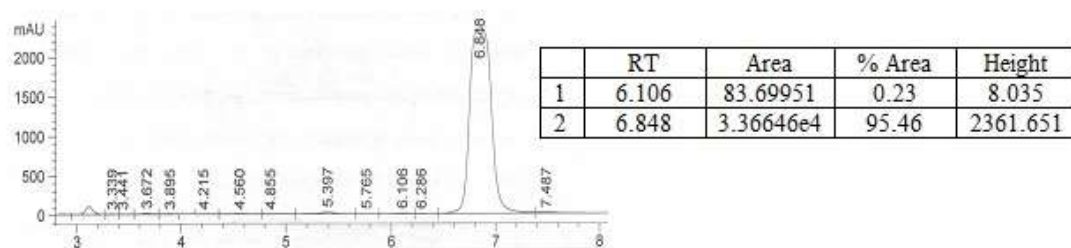
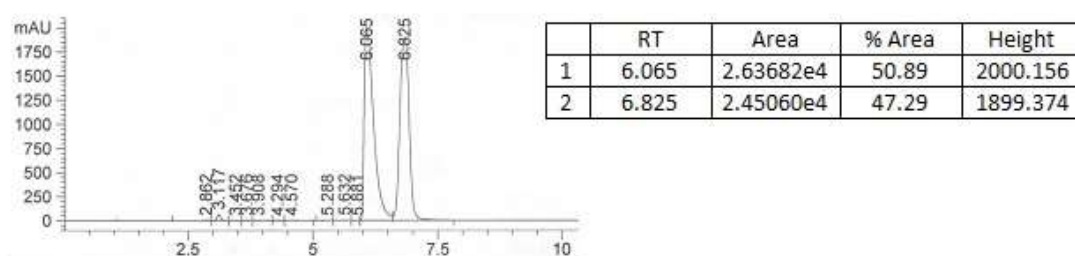
Racemic mixture of the samples was prepared chemically from the corresponding aldehydes using the same procedure. The enantiomeric excess was determined by HPLC (Agilent Technologies, HP110) with CHIRALPAK IA column (Chiral Technologies Europe, Cedex, France) at 210 nm for all samples.

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

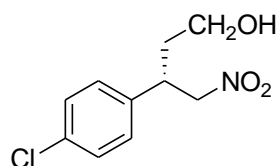


$[\alpha]_{\text{D}}^{25} = -22.0$ (c 1.0, CH_2Cl_2 , >99% ee).

HPLC conditions: heptane/*i*PrOH in the ratio of 80/20, flow rate = 1.0 ml/min ($t_{\text{r}}=6.06$ min, $t_{\text{r}}=6.83$ major), ee >99%.

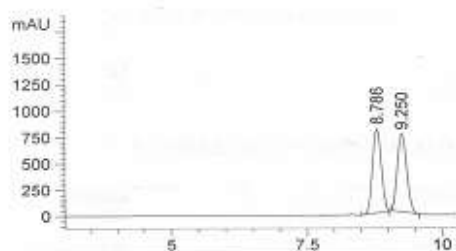


B) 3-(4-chlorophenyl)-4-nitrobutan-1-ol (obtained from biotransformation product 5)⁴

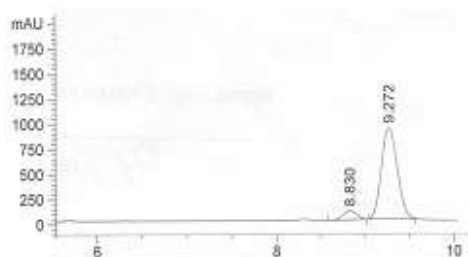


$[\alpha]_{\text{D}}^{25} = -11.5$ (c 2.0, CH_2Cl_2 , 84% ee). ^1H NMR (200 MHz): 7.35-7.27 (m, 2H); 7.19-7.14 (m, 2H); 4.71-4.52 (m, 2H); 3.77-3.56 (m, 2H); 3.51-3.39 (m, 1H);

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%.

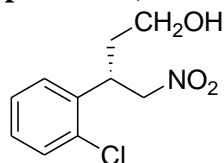


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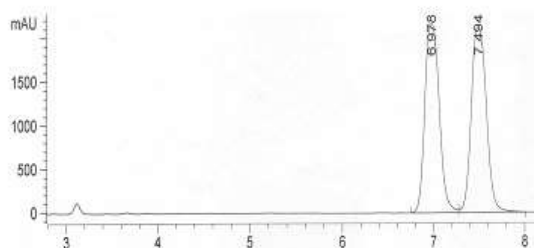


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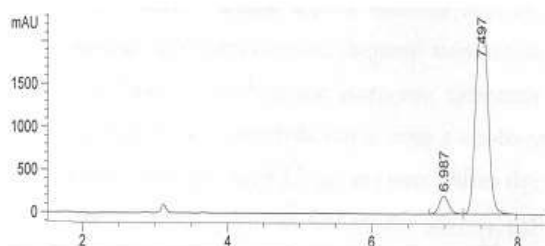
C) 3-(2-chlorophenyl)-4-nitrobutan-1-ol (obtained from biotransformation product 7)



$[\alpha]_{\text{D}}^{25} = +4$ (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%.

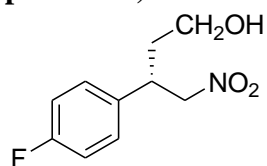


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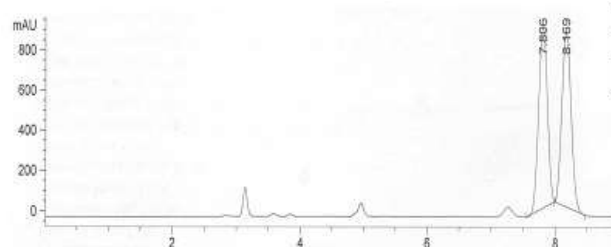
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1	6.987	1989.6008	6.964	213.228
2	7.497	2.65793e4	93.036	2205.303

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.

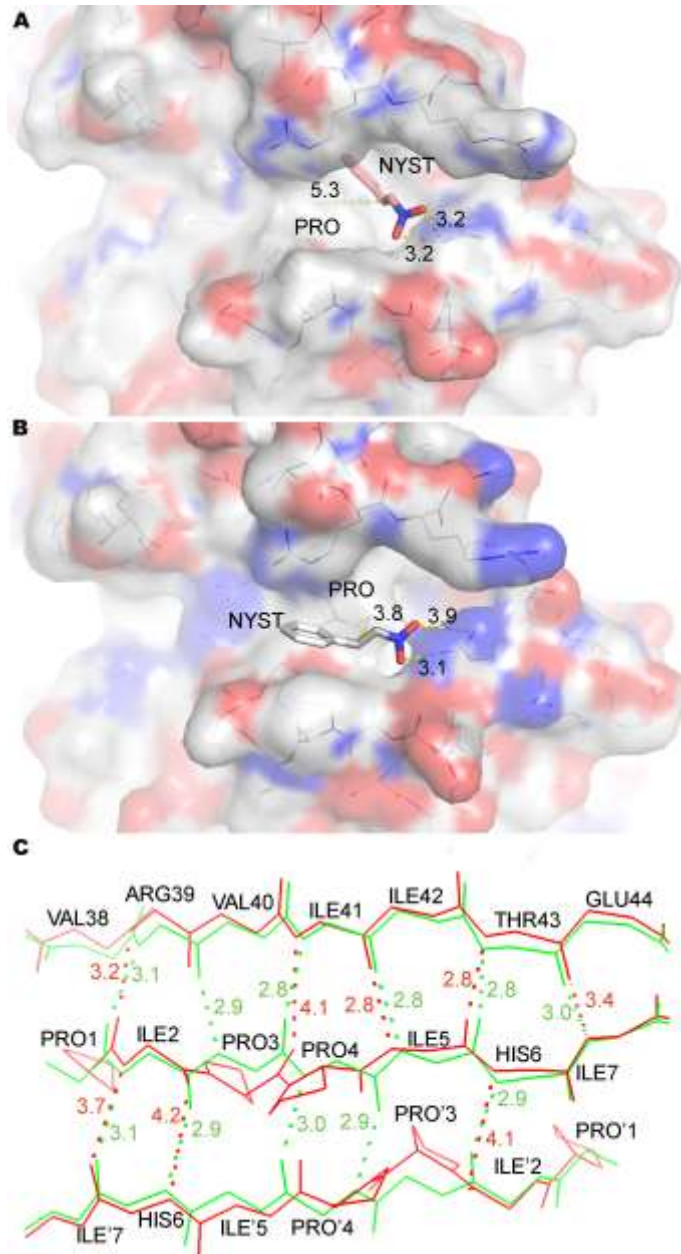
HPLC conditions: heptane/ethanol in the ratio of 80/20, flow rate = 1.0 ml/min (tr=7.81 min, tr=8.17 major), ee 94%.



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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.
2. Palomo, C.; Landa, A.; Mielgo, A., Water-compatible iminium activation: Organocatalytic Michael reactions of carbon-centered nucleophiles with enals. *Angew Chem Int Ed* **2007**, *46*, 8431-8435.
3. Jullian, V.; Quirion, J.-C.; Husson, H.-P., Enantioselective synthesis of β -substituted primary and secondary amines by alkylation of (*R*)-phenylglycinol amide enolates. *Synthesis* **1997**, *1997*, 1091-1097.
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.