Narančić, T.; Kadivojevic, J.; Jovanovic, P.; Francuski, D.; Bigović, M.; Maslak, V.; Savić, V.; Vasiljević, B.; O'Connor, K. E.; Nikodinović-Runić, J. Highly Efficient Michael-Type Addition of Acetaldehyde to Beta-Nitrostyrenes by Whole Resting Cells of Escherichia Coli Expressing 4-Oxalocrotonate Tautomerase. *Bioresource Technology* **2013**, *142*, 462–468. https://doi.org/10.1016/j.biortech.2013.05.074

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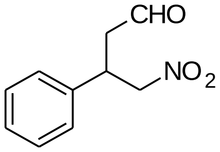
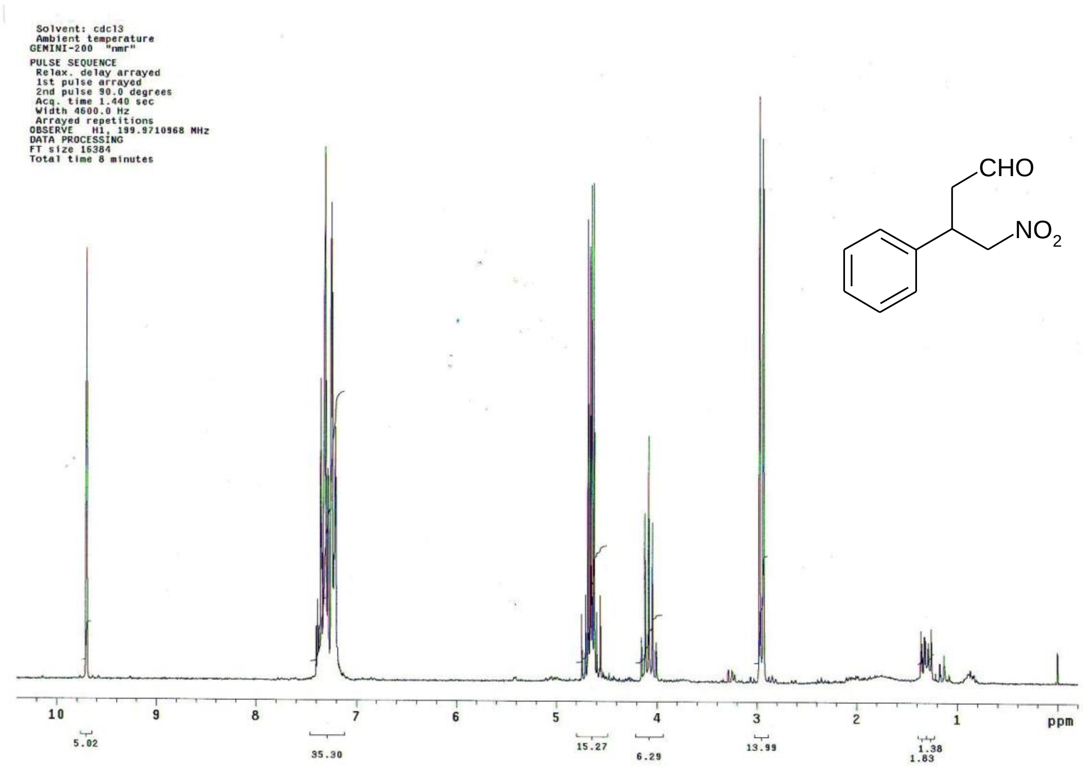
S1(A-D) Characterization data and NMR spectra of addition products obtained by biotransformation using *E.coli* BL21(4-OT)

^{1}H 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]



^{1}H 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=6.8Hz). ^{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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 Solvent: cdcl3
 Ambient temperature
GEMINI-200  "nmr"
PULSE SEQUENCE
 Relax. delay arrayed
 1st pulse arrayed
 2nd pulse 90.0 degrees
 Acq. time 1.440 sec
 Width 4600.0 Hz
 Arrayed repetitions
OBSERVE   H1, 199.9710968 MHz
DATA PROCESSING
FT size 16384
Total time 8 minutes
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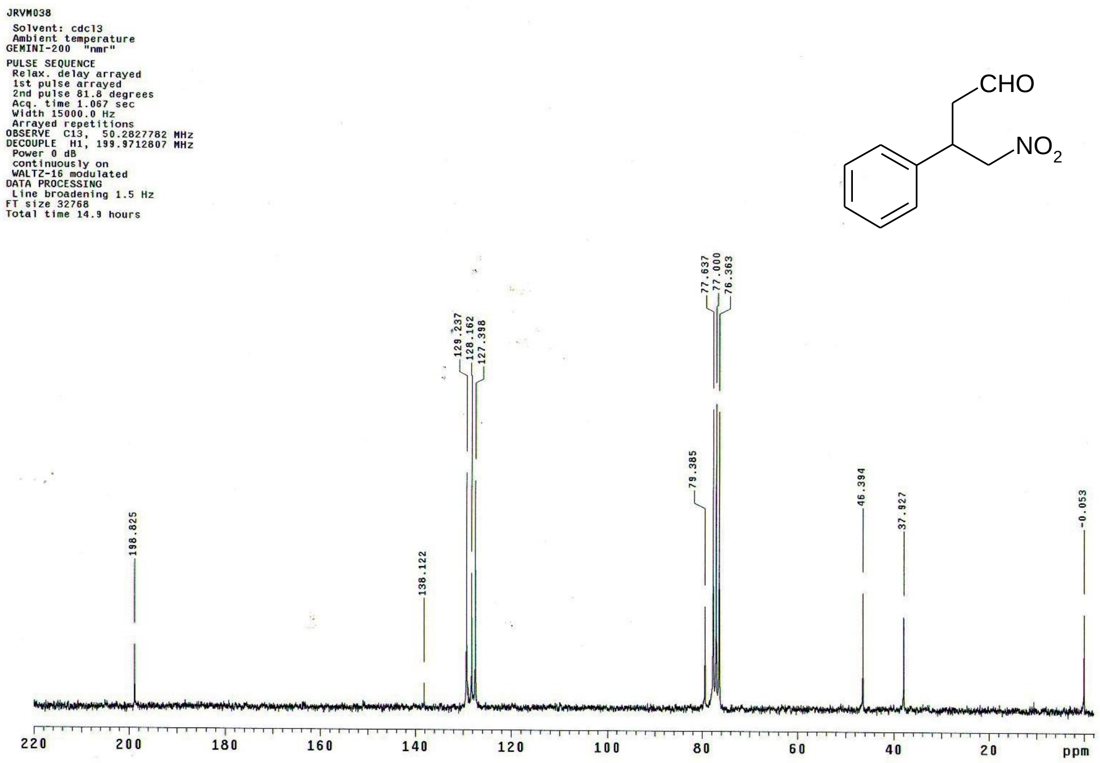

```
JRVM038
 Solvent: cdcl3
 Ambient temperature
GEMINI-200  "nmr"
PULSE SEQUENCE
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 1st pulse arrayed
 2nd pulse 81.8 degrees
 Acq. time 1.067 sec
 Width 15000.0 Hz
 Arrayed repetitions
OBSERVE  C13,  50.2827782 MHz
DECOUPLE  H1, 199.9712807 MHz
 Power 0 dB
 continuously on
 WALTZ-16 modulated
DATA PROCESSING
 Line broadening 1.5 Hz
FT size 32768
Total time 14.9 hours
```
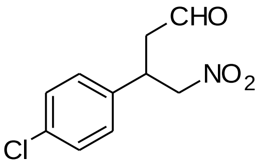
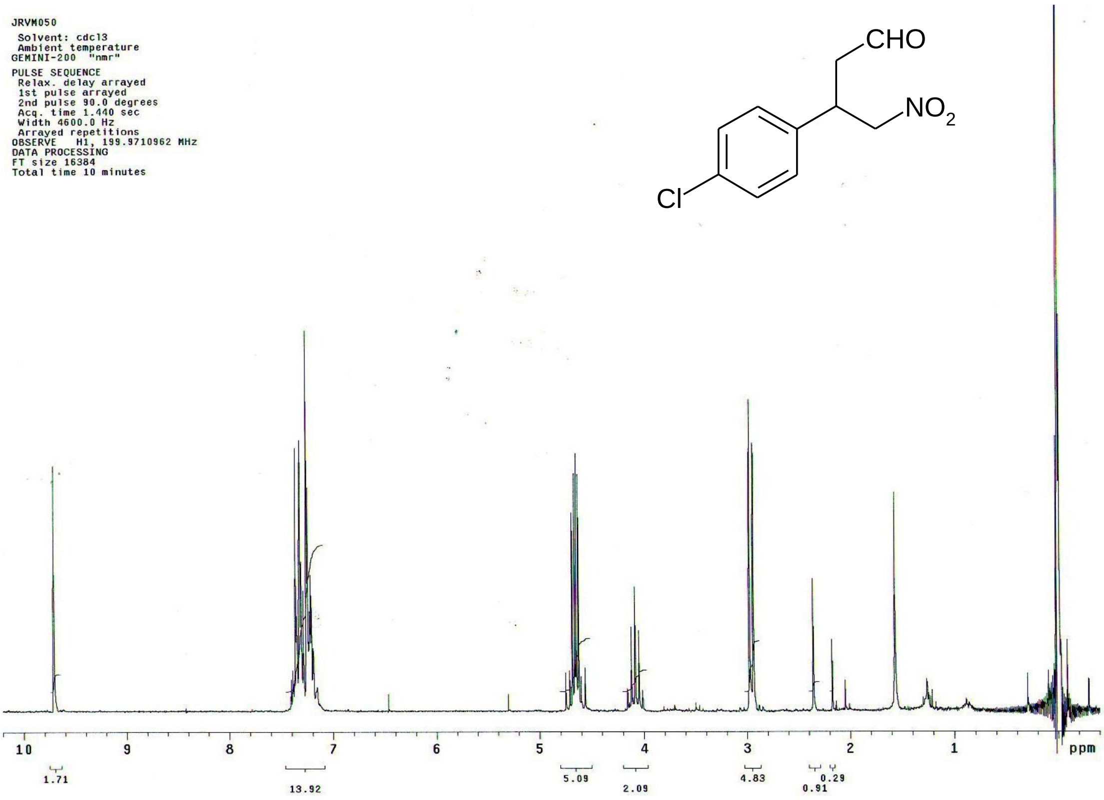

B) 3-(4-chlorophenyl)-4-nitrobutanal (5)



^{1}H 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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 Ambient temperature
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PULSE SEQUENCE
 Relax. delay arrayed
 1st pulse arrayed
 2nd pulse 90.0 degrees
 Acq. time 1.440 sec
 Width 4600.0 Hz
 Arrayed repetitions
OBSERVE   H1, 199.9710962 MHz
DATA PROCESSING
FT size 16384
Total time 10 minutes
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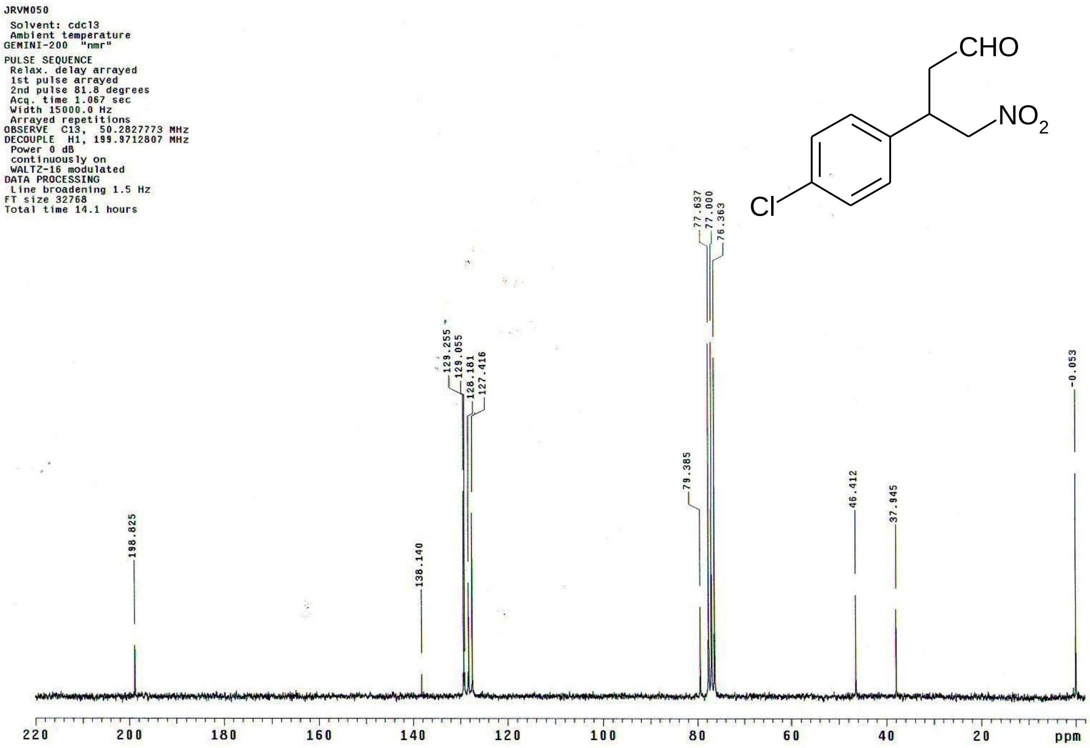
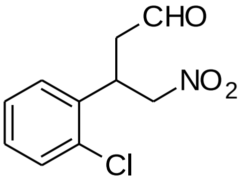
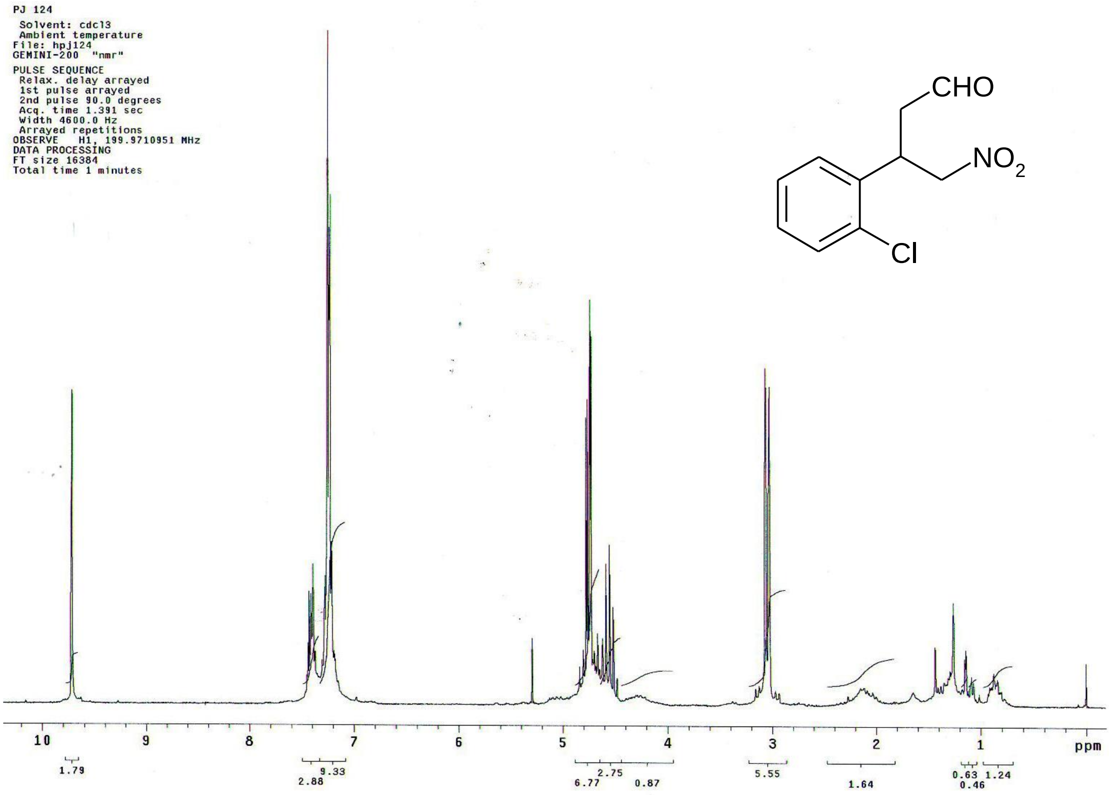


```
JRVM050
 Solvent: cdcl3
 Ambient temperature
GEMINI-200  "nmr"
PULSE SEQUENCE
 Relax. delay arrayed
 1st pulse arrayed
 2nd pulse 81.8 degrees
 Acq. time 1.067 sec
 Width 15000.0 Hz
 Arrayed repetitions
OBSERVE  C13,  50.2827773 MHz
DECOUPLE  H1, 199.9712807 MHz
 Power 0 dB
 continuously on
 WALTZ-16 modulated
DATA PROCESSING
 Line broadening 1.5 Hz
FT size 32768
Total time 14.1 hours
```

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



^{1}H 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). ^{13}C NMR (50MHz): 198.7; 135.3; 133.7; 130.5; 129.2; 128.4; 127.5; 77.3; 45.0; 34.5.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PJ 124
 Solvent: cdcl3
 Ambient temperature
File: hpj124
GEMINI-200  "nmr"
PULSE SEQUENCE
 Relax. delay arrayed
 1st pulse arrayed
 2nd pulse 90.0 degrees
 Acq. time 1.391 sec
 Width 4600.0 Hz
 Arrayed repetitions
OBSERVE   H1, 199.9710951 MHz
DATA PROCESSING
FT size 16384
Total time 1 minutes
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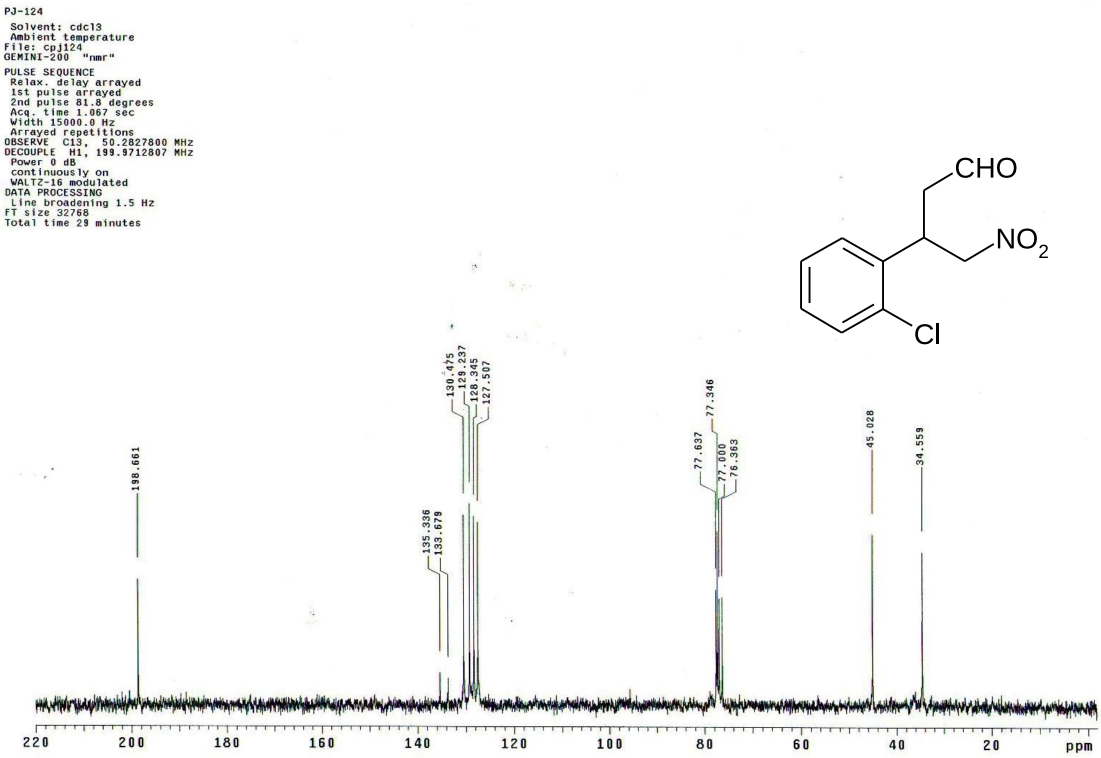

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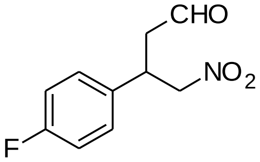
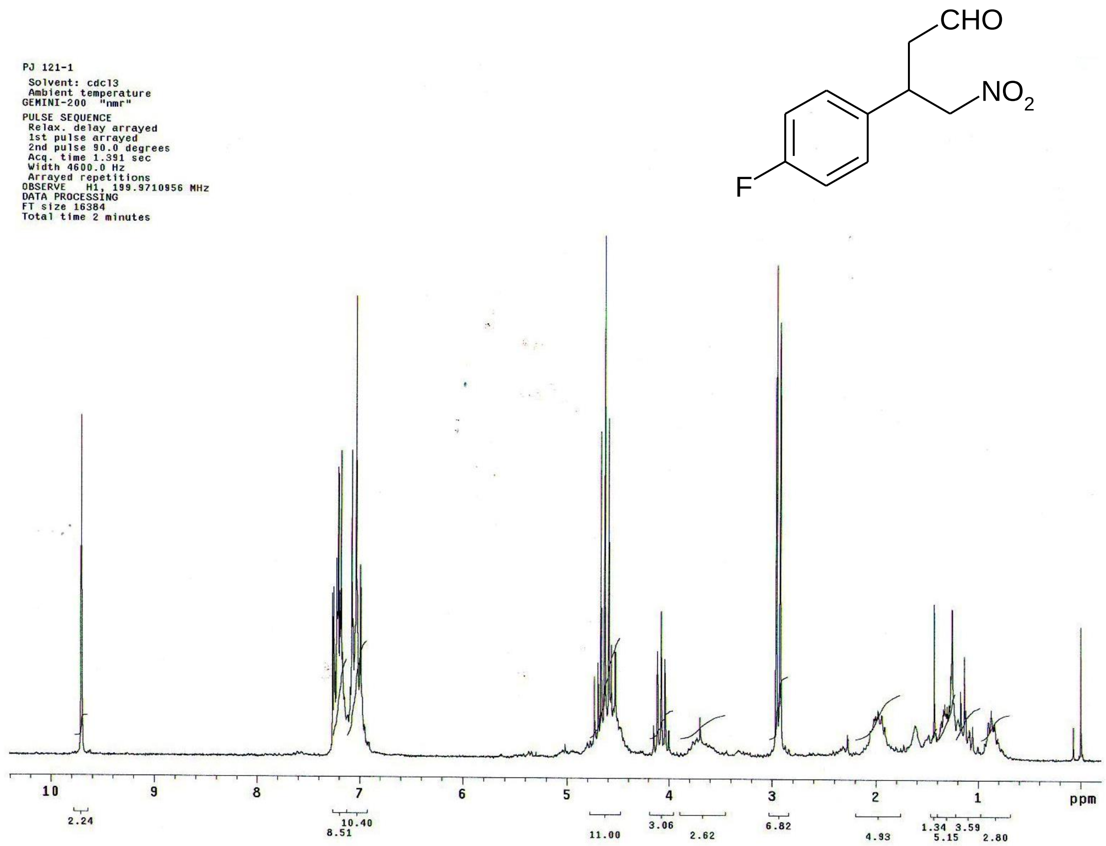
PJ-124
 Solvent: cdcl3
 Ambient temperature
File: cpj124
GEMINI-200  "nmr"
PULSE SEQUENCE
 Relax. delay arrayed
 1st pulse arrayed
 2nd pulse 81.8 degrees
 Acq. time 1.067 sec
 Width 15000.0 Hz
 Arrayed repetitions
OBSERVE  C13,  50.2827800 MHz
DECOUPLE  H1, 199.9712807 MHz
 Power 0 dB
 continuously on
 WALTZ-16 modulated
DATA PROCESSING
 Line broadening 1.5 Hz
FT size 32768
Total time 29 minutes
```

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



^{1}H 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=7.2Hz). ^{13}C NMR (50 MHz): 198.5; 133.9; 129.2; 129.0; 115.9; 79.3; 46.4; 37.2.



```
PJ 121-1
 Solvent: cdcl3
 Ambient temperature
GEMINI-200  "nmr"
PULSE SEQUENCE
 Relax. delay arrayed
 1st pulse arrayed
 2nd pulse 90.0 degrees
 Acq. time 1.391 sec
 Width 4600.0 Hz
 Arrayed repetitions
OBSERVE   H1, 199.9710956 MHz
DATA PROCESSING
FT size 16384
Total time 2 minutes
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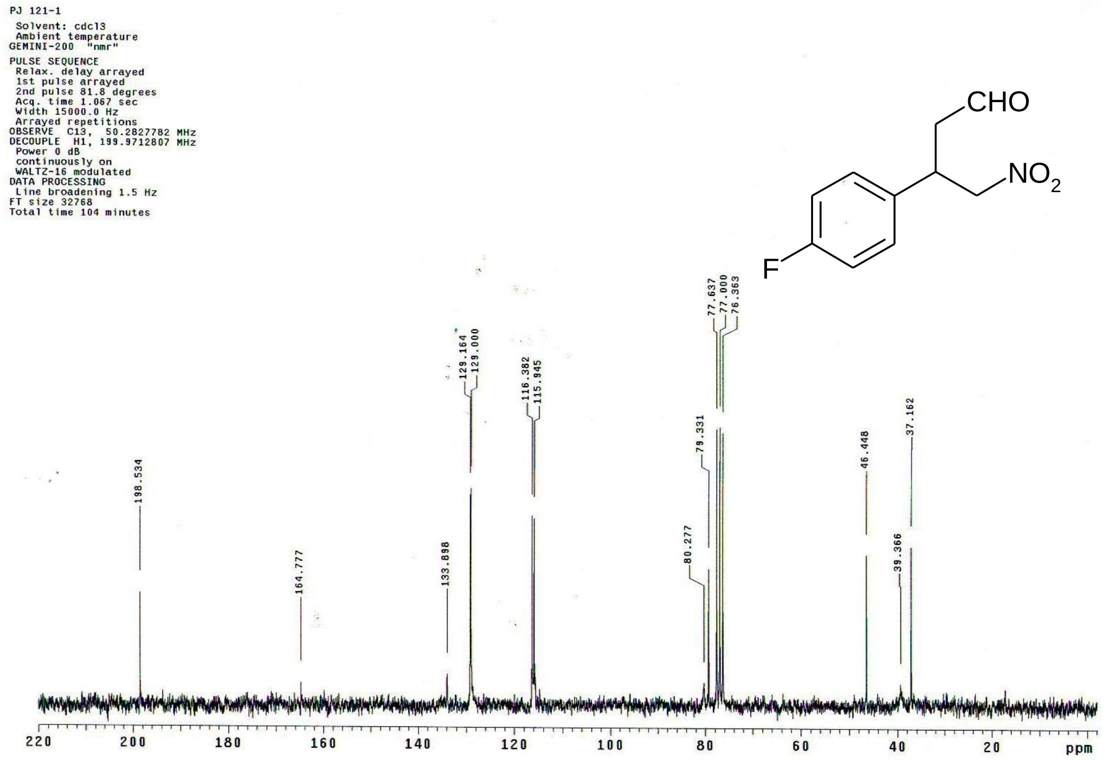


```
PJ 121-1
 Solvent: cdcl3
 Ambient temperature
GEMINI-200  "nmr"
PULSE SEQUENCE
 Relax. delay arrayed
 1st pulse arrayed
 2nd pulse 81.8 degrees
 Acq. time 1.067 sec
 Width 15000.0 Hz
 Arrayed repetitions
OBSERVE  C13,  50.2827782 MHz
DECOUPLE  H1, 199.9712807 MHz
 Power 0 dB
 continuously on
 WALTZ-16 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) $NaBH_4$ (0.15 mmol) was added at 0°C. The reaction was stirred for 30 min and quenched with H_2O and extracted with ethyl acetate (3 times 10 ml). Combined organic layers were washed with brine and dried ($MgSO_4$), 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).

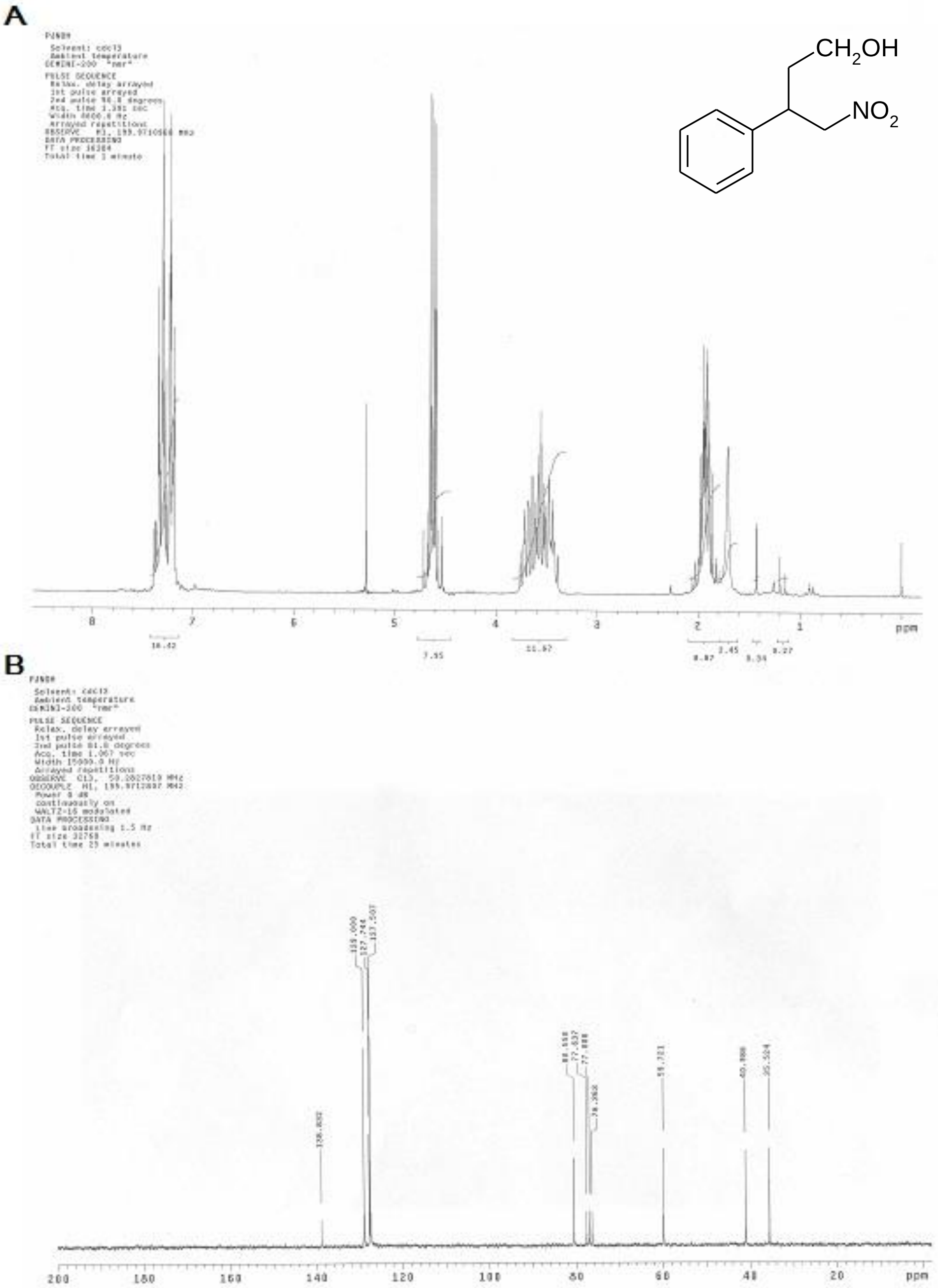


Fig. S2-A. A)^{1}H NMR spectrum of prepared 4-nitro-3-phenylbutanol (200 MHz): 7.38-7.18 (m, 5H); 4.71-4.53 (m, 2H); 3.76-3.40 (m, 3H); 2.05-1.80 (m, 2H), 1.71 (s, 1H). B) ^{13}C NMR (50 MHz): 138.8; 129.0; 127.7; 127.5; 80.5; 59.7; 41.0; 35.5.

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-phenylbtanol as viscous oil (17 mg, 99%).

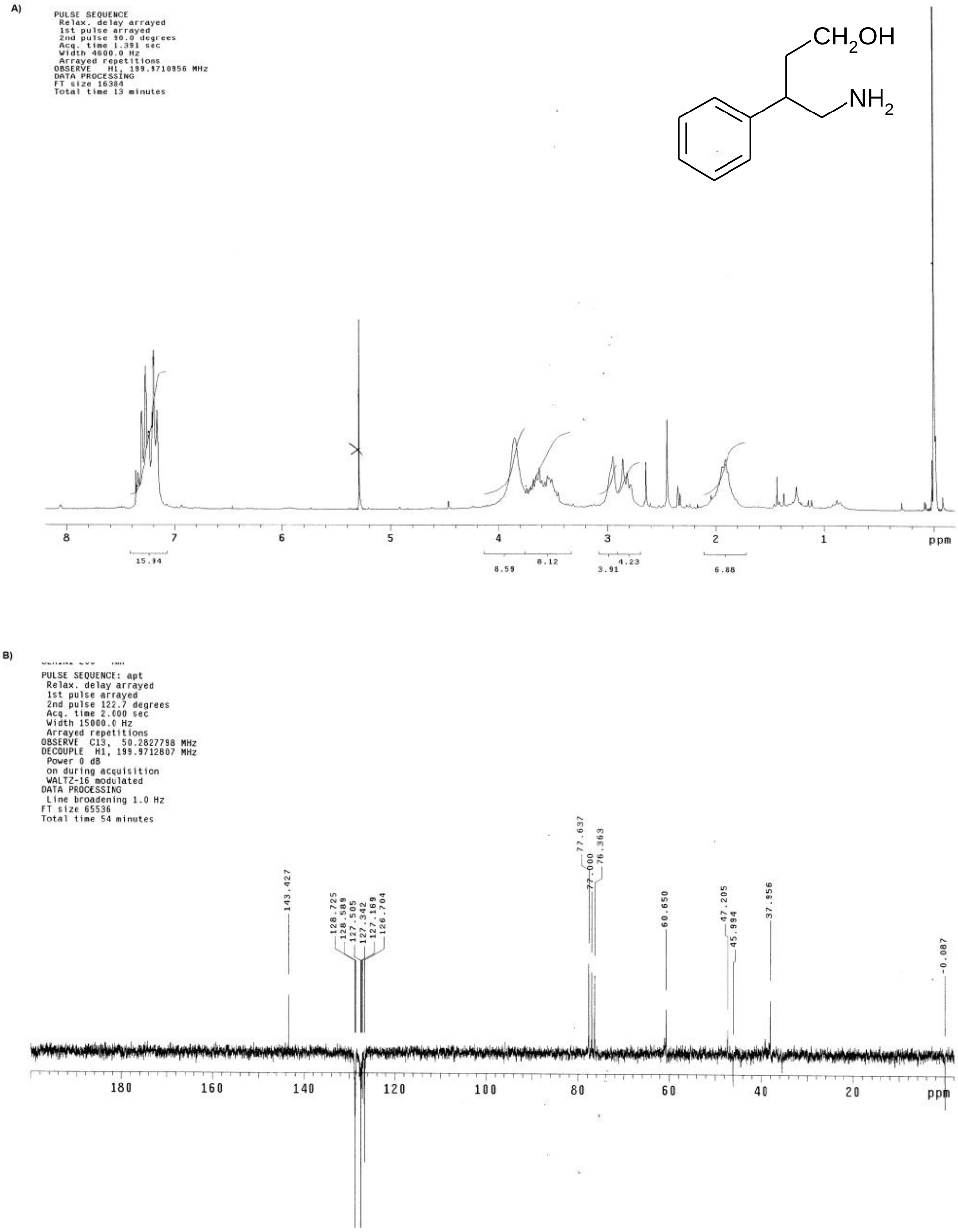
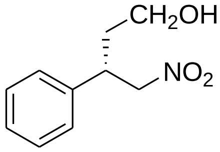


Fig. S2-B. A)^{1}H NMR spectrum of prepared 4-amino-3-phenylbutanol (**10**). B) ^{13}C NMR (50 MHz): 143.4; 128.7; 127.5; 126.7 60.7; 47.2; 46.0; 38.0.

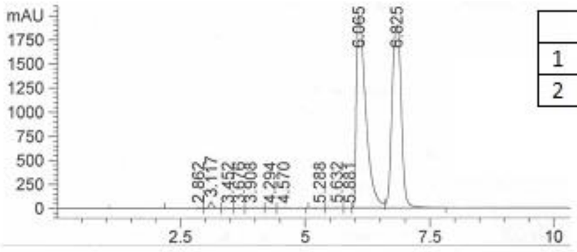
S3(A-D) Chiral analysis of biotransformation products

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.

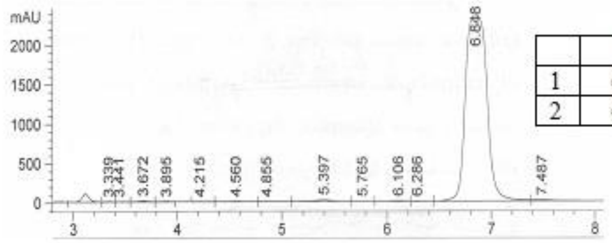
A) 4-nitro-3-phenylbutan-1-ol (obtained from biotransformation product 3)



$[\alpha]_D^{25}$ = -22.0 (c 1.0, CH_2Cl_2, >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%.

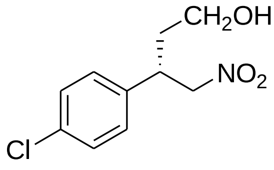


	RT	Area	% Area	Height
1	6.065	2.63682e4	50.89	2000.156
2	6.825	2.45060e4	47.29	1899.374



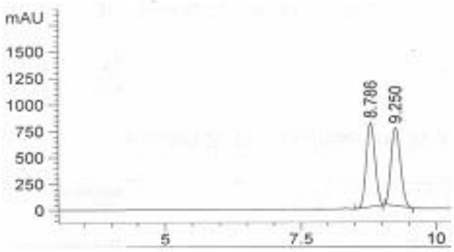
	RT	Area	% Area	Height
1	6.106	83.69951	0.23	8.035
2	6.848	3.36646e4	95.46	2361.651

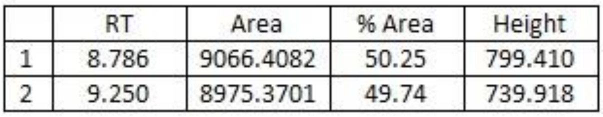
B) 3-(4-chlorophenyl)-4-nitrobutan-1-ol (obtained from biotransformation product 5) [4]



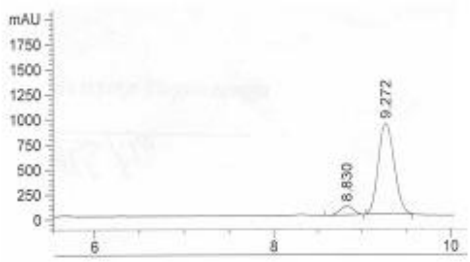
$[\alpha]_D^{25}$ = -11.5 (c 2.0, CH_2Cl_2, 84% ee). ^1H NMR (200 MHz): 7.35-7.27 (m, 2H); 7.19-7.14 (m, 2H); 4.71-4.52 (m, 2H); 3.77-3.56 (m, 2H); 3.51-3.39 (m, 1H);

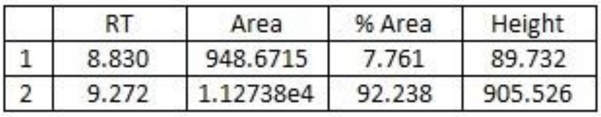
2.06-1.79 (m, 2H); 1.68 (s, 1H). ^{13}C NMR (50 MHz): 137.4; 133.5; 129.2; 128.9; 80.3; 59.5; 40.4; 35.4. HPLC conditions: heptane/ethanol in the ratio of 80/20, flow rate =1.0 ml/min (tr=8.83 min, tr=9.27 major), ee 84%.





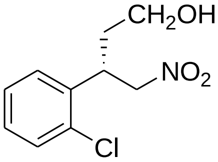
	RT	Area	% Area	Height
1	8.786	9066.4082	50.25	799.410
2	9.250	8975.3701	49.74	739.918



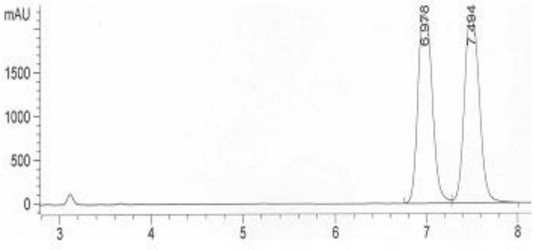


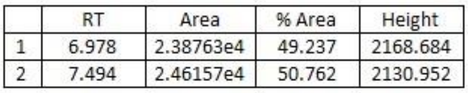
	RT	Area	% Area	Height
1	8.830	948.6715	7.761	89.732
2	9.272	1.12738e4	92.238	905.526

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

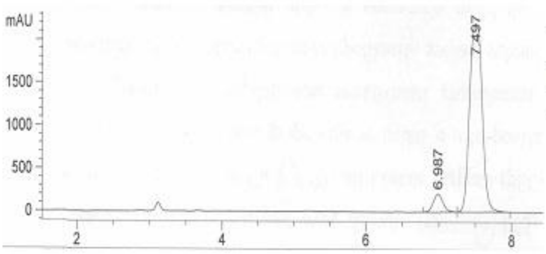


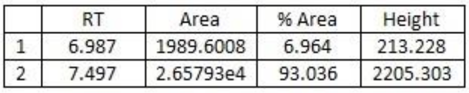
$[\alpha]_D^{25}$ = +4 (c 1.0, CH_2Cl_2, 88% ee). ^1H NMR (200 MHz): 7.44-7.39 (m, 1H); 7.28-7.18 (m, 3H); 4.81-4.64 (m, 2H); 4.36-4.21 (m, 1H); 3.69-3.49 (m, 2H); 2.08-1.98 (m, 2H); 1.60 (s, 1H). ^{13}C NMR (50 MHz): 136.3; 134.2; 130.4; 128.9; 128.2; 127.5; 78.9; 59.9; 37.3; 34.7. HPLC conditions: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





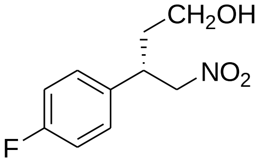
	RT	Area	% Area	Height
1	6.978	2.38763e4	49.237	2168.684
2	7.494	2.46157e4	50.762	2130.952





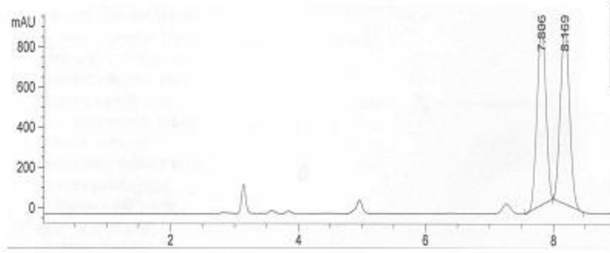
	RT	Area	% Area	Height
1	6.987	1989.6008	6.964	213.228
2	7.497	2.65793e4	93.036	2205.303

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

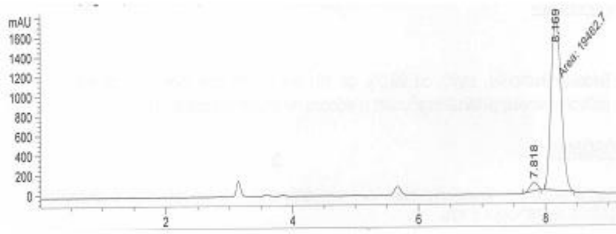


$[\alpha]_D^{25}$ = -10 (c 1.0, CH_2Cl_2, 94% ee). ^{1}H NMR (200 MHz): 7.27-7.16 (m, 2H); 7.09-6.98 (m, 2H); 4.72-4.52 (m, 2H); 3.80-3.59 (m, 2H); 3.54-3.42 (m, 1H); 2.07-1.78 (m, 2H); 1.58 (s, 1H). ^{13}C NMR (50 MHz): 137.9; 134.5; 129.2; 129.1; 80.6; 59.7; 40.3; 35.6.

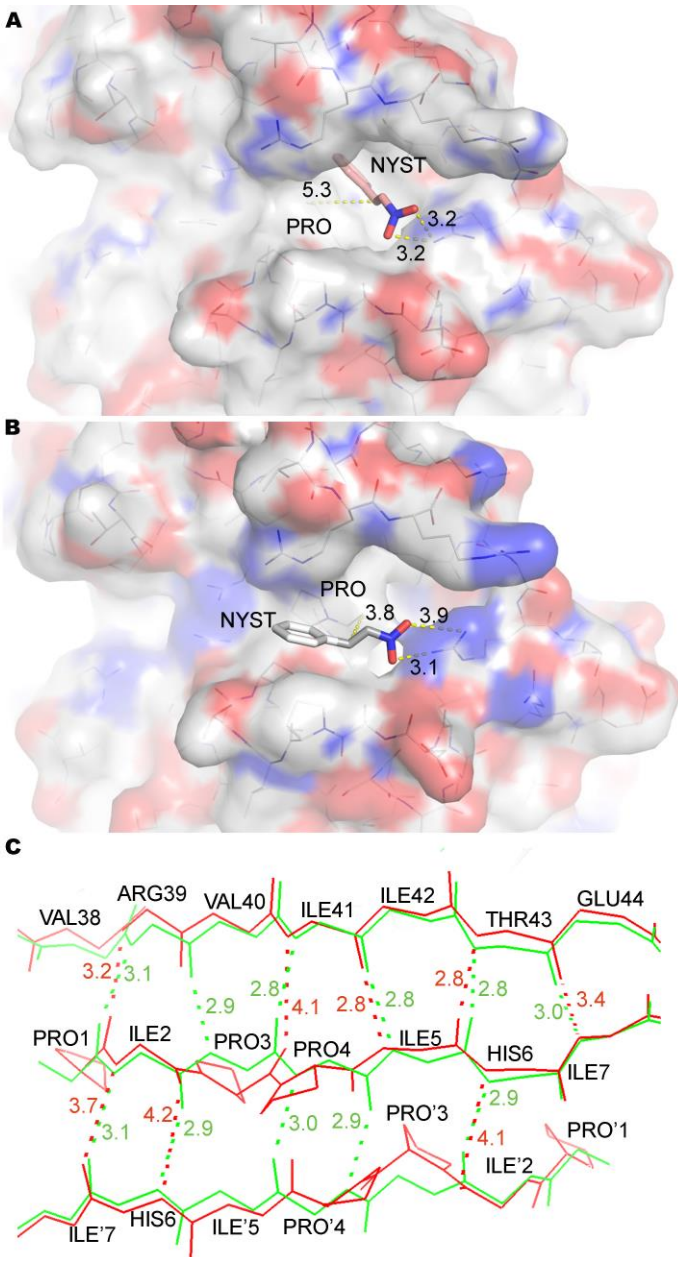
HPLC conditions: heptane/ethanol in the ratio of 80/20, flow rate =1.0 ml/min (tr=7.81 min, tr=8.17 major), ee 94%.



	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.
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.