Supporting Information

Unifying Scheme for the Biosynthesis of Acyl-Branched Sugars: Extended Substrate Scope of Thiamine-Dependent Enzymes

J.-P. Steitz, L. Krug, L. Walter, K. Hernández, C. Röhr, P. Clapés, M. Müller*
Contents

1. Enzymatic synthesis and purification of Aldol products .......................................................... 3
   Expression of hpal .................................................................................................................... 3
   Expression of MBP-Yfau .................................. .................................................................. 3
4-Hydroxy-2-oxobutanoate (22, HOBA) .................................................................................... 3
(rac)-4-Hydroxy-2-oxopentanoate (5, HOPA) ........................................................................... 4
4-Hydroxy-2-oxohexanoate (23, HOHA) .................................................................................. 6

2. Molecular biological work ......................................................................................................... 7
   Cloning of erwE, mygE, and mmar_2332: ............................................................................... 7
   E. coli BL21-Gold(DE3)::pET19b::erwE_N-His_{10} .......................................................... 7
   E. coli BL21-Gold(DE3)::pET28a::mygE_N-His_{6} ............................................................... 7
   E. coli BL21-Gold(DE3)::pET28a::mmar_2332_N-His_{6} ..................................................... 7
   Expression of erwE, mygE, and mmar_2332 .......................................................................... 7
   Purification of ErwE, MygE, and MMAR_2332 ...................................................................... 7

3. Preparative scale assays .......................................................................................................... 8
   ErwE ...................................................................................................................................... 8
   (R)-1-(4-Bromophenyl)-1,4-dihydroxybutan-2-one (25) ...................................................... 8
   (1R,4R)-1-(4-Bromophenyl)-1,4-dihydroxypentan-2-one (26a) ........................................... 9
   (1R,4R)-1-(4-Bromophenyl)-1,4-dihydroxybutan-2-one (27a) ............................................. 11
   3-Hydroxy-1-(3-hydroxytetrahydro-2H-pyran-3-yl)-butan-1-one (23) ............................ 12

   MyGE ..................................................................................................................................... 13
   (R)-1-(4-Bromophenyl)-1,4-dihydroxybutan-2-one (25) .................................................... 13
   (1R,4S)-1-(4-Bromophenyl)-1,4-dihydroxypentan-2-one (26b) ......................................... 13
   (1R,4S)-1-(4-Bromophenyl)-1,4-dihydroxybutan-2-one (27b) ............................................. 14

   MMAR_2332 ......................................................................................................................... 15
   (R)-1-(4-Bromophenyl)-1,4-dihydroxybutan-2-one (25) .................................................... 15
   (1R,4R)-1-(4-Bromophenyl)-1,4-dihydroxypentan-2-one (26a) ........................................ 15

4. Analytical scale assay: ............................................................................................................. 16
   Analytical data of products ..................................................................................................... 16

5. NMR spectra: ............................................................................................................................ 21
   ^1H NMR: ................................................................................................................................ 21
   ^13C NMR: ............................................................................................................................. 28

6. Nucleotide and amino acid sequences of ErwE and MyGE ................................................... 33

7. References ................................................................................................................................. 35
1. Enzymatic synthesis and purification of Aldol products

Enzymatic aldolase reactions were used to synthesize 4-hydroxy-2-oxoacids, that were tested as substrates with ErwE and MyGE. The plasmid for the expression of hpal was provided by Dr. Seah, University of Gulph.

Expression of hpal

Hpal was expressed in 300 mL of LB media, supplemented with 1.0 mM D-sorbitol and 2.5 mM glycine betaine in a 2 L flask. The expression of hpal was induced at an OD$_{600}$ = 0.6 with 0.5 mM IPTG. The cells were harvested at 18600 × g for 20 minutes, resolved in assay buffer (50 mM KPi; 155 mM NaCl; pH = 8), and disrupted by sonication. After 90 minutes of centrifugation at 12600 × g the supernatant was used for the preparative scale enzymatic assays.

Expression of MBP-Yfau

Cloning of 2-keto-3-deoxy-L-rhamnionate aldolase (YfaU EC 4.1.2.53) from E. coli K-12 in pQE40-MBP plasmid was carried out as described in our previous publications. Production of MBP-YfaU was performed as follows. Plasmid pQE40-MBP-YfaU was transformed into M-15[pREP-4] E. coli strain from QIAGEN and grown in LB medium with ampicillin (100 µg mL$^{-1}$) plus kanamycin (25 µg mL$^{-1}$) at 37 ºC, on a rotary shaker at 200 rpm. A final optical density at 600 nm (OD$_{600}$) of 2–3 was usually achieved. An aliquot of the pre-culture (12 mL) was transferred into a shake-flask (2 L) containing LB (600 mL), ampicillin (100 µg mL$^{-1}$), kanamycin (25 µg mL$^{-1}$) and incubated at 37 ºC with shaking at 200 rpm. During the middle exponential phase growth (OD$_{600}$ ≈ 0.5–0.8), the temperature was decreased to 20 ºC to minimize potential inclusion bodies formation and isopropyl-β-D-1-thiogalactopyranoside (IPTG; 50 µM final concentration) was added (after 12 h, OD$_{600}$ = 6.4–7.0). Cells from the induced-culture broths (3 L, 4.3–5.3 g of cells L$^{-1}$ of medium) were centrifuged (12 000 g for 30 min at 4 ºC). The pellet was resuspended in starting buffer (200 mL) consisting of 50 mM sodium phosphate buffer pH 8.0, containing NaCl (300 mM) and imidazole (10 mM). Cells were lysed using a cell disrupter. Cellular debris was removed by centrifugation at 30 000 g for 30 min. The clear supernatant was collected and purified by immobilized metal ion affinity chromatography (IMAC) using a FPLC system (Amersham biosciences). The crude supernatant was applied to a cooled HR 16/40 column (GE Healthcare) packed with HiTrap chelating support (50 mL bed volume; Amersham Biosciences) and washed with starting buffer (250 mL). The protein was eluted with 50 mM sodium phosphate buffer pH 8.0, containing NaCl (300 mM) and imidazole (500 mM) at 3 mL min$^{-1}$. Fractions containing the recombinant protein were dialyzed against 2 mM MOPS buffer pH 7.0 at 4 ºC. The dialyzed solution was frozen at −80 ºC and lyophilized. The white solid obtained was stored at −20 ºC. Protein concentration (0.5 mg protein per mg of lyophilized solid) was determined using the Bradford protein assay in 96 wells plate.
4-Hydroxy-2-oxobutanoate (22, HOBA)

The reaction was conducted as previously described. Reaction (7.7 mL total volume) was conducted in a Falcon Tube (15 mL). MBP-YfaU-Mg\(^{2+}\) (15.4 mg of protein, 2 mg mL\(^{-1}\), 77 U, 10 U mL\(^{-1}\)) was dissolved in sodium pyruvate (1) solution (7.1 mL of a 1.0 M stock solution at pH 6.5–7.0, adjusted with NaOH, 50 mM, 1 M in the reaction). The reaction was initiated by addition of formaldehyde (626 µL of a 12.3 M commercial aqueous solution, 1 M in the reaction) and shaken in a vortex mixer (1000 rpm) at 25 °C. After 16 h, the reaction was centrifuged (5000 g at 4 °C for 30 min) and the MBP-YfaU in the supernatant was eliminated using an Amicon ultrafiltration unit (Millipore, USA, MWCO 10 kDa, 5000 g at 4 °C for 60 min). Solution was frozen at –80 °C and lyophilized to afford the title compound as a white solid (980 mg, 98% containing ca 4-hydroxy-3-(hydroxymethyl)-2-oxobutanoate (10%) as a result of the addition of 22 to a second equivalent of formaldehyde). Sodium 4-hydroxy-2-oxobutanoate (22) The spectral properties of this product agreed with those reported in a previous publication.\(^{[3]}\)

\(^1\)H NMR (400 MHz, D\(_2\)O) δ 3.71 (t, J = 5.9 Hz, 2H), 2.88 (t, J = 5.9 Hz, 2H).

\(^{13}\)C NMR (101 MHz, D\(_2\)O) δ 200.56 (CO), 166.37 (CO\(^{-2}\)), 55.94 (CH\(_2\)OH), 41.14 (CH\(_2\)).

Sodium 2,2,4-trihydroxybutanoate (hydrate form of 22):

\(^1\)H NMR (400 MHz, D\(_2\)O) δ 3.54 (t, J = 6.7 Hz, 2H), 1.95 (t, J = 6.7 Hz, 2H).

\(^{13}\)C NMR (101 MHz, D\(_2\)O) δ 174.44 (CO\(^{-2}\)), 118.85 (C(OH)\(_2\)), 56.77 (CH\(_2\)OH), 40.14 (CH\(_2\)).

Sodium 4-hydroxy-3-(hydroxymethyl)-2-oxobutanoate:

\(^1\)H NMR (400 MHz, D\(_2\)O) δ 3.72 (m, 4H), 3.33 (m, 1H).

\(^{13}\)C NMR (101 MHz, D\(_2\)O) δ 58.56 (CH\(_2\)OH), 52.41 (CH).
(rac)-4-Hydroxy-2-oxopentanoate (5, HOPA)

1.2 mL Acetaldehyde (aqueous solution, 1 M, 1.2 mmol) and 1.3 g sodium pyruvate (1) (0.65 M, 11.8 mmol) were added to 20 mL supernatant of crude cell extract (E. coli expression culture of hpal) supplemented with 1 mM CoCl₂. The assay was slowly stirred in a round-bottom flask at room temperature. After 16 hours 2 mL of NaOH (5 M) were added to deprotonate the ketoacid. The enzyme was precipitated by addition of ethyl acetate and filtered off. The reaction catalyzed by the aldolase is reversible, thus product formation was monitored using ¹H NMR analysis to identify the time point giving the best rate of product formation. HOPA (5) was purified using an Isolera (Biotage) chromatography system equipped with a Dowex 1X8-200 anionic exchange column (20 x 70 mm).[4] After precipitation and filtration, the aqueous phase was manually loaded to the column with a syringe. After a washing step with water the product (5) was eluted using a NaCl gradient. The elution of HOPA was detected at 210 and 220 nm.

Elution with sodium chloride: Elution was performed with a gradient over 8 column volumes (CV) reaching 0.5 M NaCl. Fractions containing the product were lyophilized. After lyophilization the water-soluble residue (a mixture of sodium salt of the ketoacid and sodium chloride) was used to perform all assays with HOPA as donor substrate. All assays under standard conditions were performed using a single batch of HOPA.

¹H NMR (400 MHz, H₂O/D₂O): δ = 1.12 ppm (d, 3J = 6.3 Hz, 3H, CH₃), 2.81 (d, 3J = 6.3 Hz, 2H, CH₂), 4.19 (“sext”): 3J = 6.3 Hz; 1H; COH).

Elution with hydrogen chloride: Elution with hydrogen chloride was used to determine the enantiomeric ratio after lactonization of the product. The procedure was adapted from Lane and Dekker, describing the synthesis and purification of 4-hydroxy-2-oxobutyrate.[5] Here, the washing step with water was followed by elution with a gradient of 8 CV reaching a concentration of 0.2 M hydrogen chloride in the aqueous elution fractions (pH ~1). Product-containing fractions were combined, concentrated to a volume of 25 mL (by lyophilization), and extracted five times with an excess of dry diethyl ether (50 mL). The combined organic phases were dried over Na₂SO₄. The lactonized product 39 crystallized after evaporation of the solvent.

NMR spectroscopic data of the product compare well with the data from literature (measured in CDCl₃).[6]

¹H NMR (400 MHz, D₂O): δ = 1.27 ppm (d; Hz, 3J = 6.7 Hz; 1H; CH₃), 5.02 (dq; 3J = 6.8, Hz, 3J = 1.9 Hz; 1H; CHCH₃), 6.31 (d; 3J = 1.9 Hz; 1H; CHCOH).

¹³C NMR (100 MHz, D₂O): δ = 18.5 ppm (CH₃), 77.0 (CHCH₃), 123.6 (C=CH), 140.9 (COH), 175.9 (C=O).

GC-MS: tᵣ = 5.68 min; m/z (%) = 114 (32) [M]+, 99 (21) [C₄H₆O₅]+, 86 (4) [C₄H₅O₂]+, 69 (100), 58 (74).

C₅H₆O₃: 114.03 g·mol⁻¹

The separation of the enantiomers was established via chiral-phase HPLC column. It was shown that Hpal gives (almost) racemic HOPA (ee <5%).

HPLC (Chiralcel OB, 25 °C, 1 mL·min⁻¹, n-hexane/2-propanol = 97:3): tᵣ = 26.45 min (R), tᵣ = 30.27 min (S); ee <5%.
4-Hydroxy-2-oxohexanoate (23, HOHA)

\[
\begin{align*}
&\text{O}_2\text{C} - \text{O} \quad + \quad \text{O} \quad \text{H} \\
&\text{H}_2\text{C} - \text{CH}_3 \\
&\text{H}_2\text{C} - \text{COH} \\
&\text{O}_2\text{C} - \text{OH}
\end{align*}
\]

Sodium pyruvate (1) (1.3 g, 12 mmol, 0.65 M) and propanal (2.5 mL, 35 mmol, 1.75 M) were added to 20 mL supernatant of crude cell extract (E. coli expression culture of hpaI), supplemented with 1 mM CoCl₂. The reaction mixture was slowly stirred in a round-bottom flask at room temperature for 13 h. NaOH solution (2 mL, 5 M) was added to deprotonate the carboxylic acids in the assay. The enzyme was precipitated by addition of 5 mL ethyl acetate and removed by filtration. 4-Hydroxy-2-oxohexanoate (23) was purified by automated flash column chromatography on Dowex 1X8-200 mesh.

Elution with aqueous sodium chloride solution:

After the column was washed with five CV of water, the elution was performed with a gradient (12 CV) reaching a concentration 0.5 M NaCl in the aqueous elution fractions. Fractions containing product were identified by \(^1\)H NMR spectroscopy, combined, and lyophilized. Removal of sodium pyruvate was not fully achieved.

The lyophilisate is a mixture of the sodium 4-hydroxy-2-oxohexanoate (23) and NaCl. It is soluble in water and was used to perform all assays with 23 as donor substrate. The ratio of NaCl and sodium 4-hydroxy-2-oxohexanoate was not determined; one single batch of 23 was used for the enzymatic assays with this donor substrate.

\(^1\)H NMR (400 MHz, D₂O): \(\delta = 1.06 \text{ ppm (t; } ^3J = 7.5 \text{ Hz; } 3\text{H; } \text{CH}_3), 1.64–1.74 \text{ (m; } 2\text{H; } \text{CH}_2\text{CH}_3), 3.03 \text{ (dd; } ^2J = 16.7 \text{ Hz, } ^3J = 8.6 \text{ Hz; } 1\text{H; } \text{COCH}_2\text{H}_2), 3.14 \text{ (dd; } ^2J = 16.7 \text{ Hz, } ^3J = 4.3 \text{ Hz; } 1\text{H; } \text{COCH}_2\text{H}_2), 4.18–4.25 \text{ (m; } 1\text{H; } \text{CH}_2\text{OH}).

\(^{13}\)C NMR (100 MHz, D₂O): \(\delta = 9.3 \text{ ppm (CH}_3\text{), 29.5 (CH}_2\text{CH}_3\text{), 46.2 (COCH}_2\text{), 68.8 (CHOH), 169.8 (CO}_2\text{H), 205.4 (C=O).}

Elution with aqueous hydrogen chloride solution:

A washing step with water was followed by an elution with a gradient of 8 CV reaching a concentration of 0.2 M hydrogen chloride in the aqueous elution fractions. The fractions containing \(\alpha\)-keto acids were combined and concentrated to a volume of 12 mL by lyophilization. The acidic fractions were extracted five times with an equal amount of dry diethyl ether and subsequently dried over Na₂SO₄. Lactone 40 crystallized after evaporation of the solvent.

\[^1\)H NMR (400 MHz, CDCl₃): \(\delta = 1.00 \text{ ppm (t; } ^3J = 7.4 \text{ Hz; } 3\text{H; } \text{CH}_3), 1.67–1.84 \text{ (m; } 2\text{H; } \text{CH}_2\text{), 4.91 (m; } 1\text{H; } \text{CHCH}_2\text{), 6.21 (d; } ^3J = 2.0 \text{ Hz; } 1\text{H; } \text{CHCOH}).

\[^{13}\)C NMR (100 MHz, CDCl₃): \(\delta = 9.0 \text{ ppm (CH}_3\text{), 27.4 (CH}_2\text{), 80.8 (CHCH}_2\text{), 118.7 (CHCOH), 142.3 (COH), 170.5 (C=O).}

GC-MS: \(t_r = 6.77 \text{ min; m/z (%)} = 128 (23) [M]^+; 99 (69) [C}_4\text{H}_6\text{O}_3]^+, 83 (100), 72 (65), 57 (39), 55 (37).

\(\text{C}_6\text{H}_8\text{O}_3\): 128.05 g·mol⁻¹
2. Molecular biological work

Cloning of erwE, mygE, and mmar_2332:

*E. coli* BL21-Gold(DE3)::pET19b::erwE_N-His$_{10}$

The gene cluster harbouring *erwE* was identified in *Pectobacterium atrosepticum* SCR 1043. The sequence of the ThDP-dependent enzyme was compared in 5 different strains of *Pectobacterium atrosepticum*. The protein sequence was identical in 3 different strains. The DNA sequence of one of those strains (*P. atrosepticum* NCPPB 549) was codon-optimized for *E. coli* and synthesized by Invitrogen. PCR was used to add 15 bp overlaps on 5’ and 3’ ends of the gene, homologous to pET-19b. Infusion HD cloning kit was used for cloning and the plasmid was transformed into *E. coli* stellar competent cells.[7] The correct cloning of the vector was confirmed by sequencing. The plasmid was transformed to *E. coli* BL21-Gold(DE3) competent cells for expression.

*E. coli* BL21-Gold(DE3)::pET28a::mygE_N-His$_{6}$

The sequence of *mygE* was identified from a shotgun sequencing of *Mycobacterium gastri* 'Wayne'. It was codon-optimized using *GeneOptimizer* from GeneArt and ordered as synthetic gene at Thermo Fischer Scientific. The gene, containing 15 bp overlaps on both ends, was provided in a pMA-T vector. It was cloned into pET-28a using Ndel and XhoI. After isolation the correct sequence was confirmed by sequencing and the plasmid was transformed to *E. coli* BL23-Gold(DE3) competent cells for expression.

*E. coli* BL21-Gold(DE3)::pET28a::mmar_2332_N-His$_{6}$

*mmar* synth was cloned into pET-28a. DNA was ordered with 15 bp overlaps, complementary to pET-28a; the vector was linearized with the restriction enzymes Ndel and XhoI. The ligation of both fragments was performed with In-Fusion HD Cloning Plus Kit.

Expression of erwE, mygE, and mmar_2332

All strains for expression experiments were cultivated in 300 mL of auto-induction media, supplemented with thiamine chloride hydrochloride (10 mM) and kanamycin or ampicillin.[8] The cells were shaken in a 2 L flask at 24 °C and 160 rpm for 16 to 20 hours and harvested at 18600 × g for 15 minutes. After uptake in binding or desalting buffer (8 mL buffer per gramm wet cell weight) the cells were sonicated ten times for 15 seconds with 15 seconds breaks in between. After 90 minutes of centrifugation at 12 600 × g the supernatant was applied to IMAC.

Purification of ErwE, MygE, and MMR_2332

All three enzymes were purified by automated Ni-NTA affinity chromatography (NGC Chromatography System, Bio-Rad Laboratories, Hercules, USA) using His 60 Ni Superflow columns (5 mL; Takara, Mountain View, USA) and the software ChromLab (Version 3.3.0.09, Standard Edition, Bio-Rad Laboratories, Hercules, USA). The samples were supplied to a column with a flow rate of 2.5 mL min$^{-1}$. The purification steps were carried out at a flow rate of 5 mL min$^{-1}$ and a temperature of 8 °C. At least one washing step was performed to elute non-specifically bound proteins. The His-tagged proteins were eluted with desalting buffer (KP, 50 mM, NaCl 250 mM, MgCl$_2$ 1 mM, ThDP 50 μM; pH = 7.4) which in addition contained 500 mM imidazole. Protein containing fractions were concentrated by ultrafiltration (Vivaspin 20, 30.000 nominal molecular weight limit, Sartorius, Göttingen, Germany), before they were desalted by gel filtration (HiTrap Desalting Columns: 5 mL, four connected columns or HiPrep 26/10 Desalting column 52 mL. GE Healthcare, Chalfont St Giles, UK). Desalting buffer (without imidazole) was used for the desalting steps. Protein concentration was determined by integration of the UV absorbance measured at 280 nm, using the extinction coefficient (calculated with ProtParam[9] and the software ChromLab). Enzyme assays were performed in desalting buffer, if not stated otherwise.
3. Preparative scale assays

Preparative scale assays were performed in round-bottom flasks of appropriate size and stirred with a magnetic device at room temperature, if not stated otherwise. Assays with ErwE were run in desalting buffer [KPi (50 mM), NaCl (250 mM), MgCl₂ (1 mM), ThDP (50 μM), pH = 7.4].

**ErwE**

(R)-1-(4-Bromophenyl)-1,4-dihydroxybutan-2-one (25)

[Chemical structure diagram]

Aldehyde 24 (120 mg, 0.65 mmol, 26 mM) and α-keto acid 22 (182 mg, 1.3 mmol, 52 mM) were added to a solution of 2 mL DMSO and filled up to a volume of 20 mL with desalting buffer [KPi (50 mM), NaCl (250 mM), MgCl₂ (1 mM), ThDP (50 μM), pH = 7.4]. Freshly purified enzyme ErwE (10 mg) were added in 5 mL desalting buffer. The reaction mixture was slowly stirred under nitrogen atmosphere for 16 h and extracted three times with 25 mL ethyl acetate. After evaporation of the solvent, 245 mg of crude product was obtained and subsequently purified by automated flash column chromatography (Isolera Prime, SNAP Ultra 10 g column, Biotage, Sweden; elution with ethyl acetate/cyclohexane 10:90 (1 CV), gradient 10:90 → 100:0 (12 CV), flow rate: 12 mL·min⁻¹). 18.1 mg crystalline 25 (69 µmol, yield = 10.6%) were obtained. The CD spectrum showed a negative band at 285 nm, indicating formation of (R)-25. (R)-configuration of 25 was verified by X-ray structural analysis.

R₁: 0.34 (cyclohexane/ethyl acetate = 1:1)

^1H NMR (400 MHz, CDCl₃): δ = 2.54 ppm (ddd; 2J = 17.9, Hz, 3J = 6.1 Hz, 4J = 4.5 Hz; 1H; CH₂H₂), 2.68 (ddd; 2J = 17.9, Hz, 3J = 6.9 Hz, 4J = 5.0 Hz; 1H; CH₂H₂), [3.83 (ddd; 2J = 11.2, Hz, 3J = 9.4 Hz, 4J = 4.8 Hz; 1H; CH₂H₂OH), 3.83 (ddd; 2J = 22.4, Hz, 3J = 11.4 Hz, 4J = 4.6 Hz; 1H; CH₂H₂OH) the protons are isochrone], 5.10 (s; 1H; CHOH), AA'BB' system: centers at δA = 7.22 (2H, Hₐar) and δB = 7.53 ppm (2H, Hₐar).

^13C NMR (100 MHz, CDCl₃): δ = 40.1 ppm (CH₂), 57.5 (CH₂OH), 79.4 (CHOH), 123.0 (Cₗar), 129.0 (2C, CHₐar), 132.2 (2C, CHₐar), 136.5 (CBrₐar), 209.0 (C=O).

GC-MS: tR = 12.19 min; m/z (%) = 240 (1), 230 (8), 187 (69) 185 (100) [C₇H₇BrO⁺], 183 (30), 157 (21), 133 (2), 105 (5) 77 (40).

CD (CH₃CN): λ (Δεrel.) = 300 nm (−39), 285 (−100), 270 (−67).

HPLC (Chiralpak AS-H, 25 °C, 0.95 mL·min⁻¹, n-hexane/2-propanol = 92:8): tR = 20.34 min (R), tₘ = 18.96 min (S); ee >95% (standard assay conditions, 20 h incubation).

C₁₀H₁₁BrO₃: 258.99 g·mol⁻¹
(1R,4R)-1-(4-Bromophenyl)-1,4-dihydroxypentan-2-one (26a)

\[
\begin{align*}
\text{OH} & \quad \text{CO}_2^- \\
\text{CO}_2^- & \quad \text{OH} \\
\text{Br} & \quad \text{OH}
\end{align*}
\]

Aldehyde 24 (157 mg, 0.84 mmol, 34 mM) and racemic 5 (550 mg) were added to 5 mL DMSO and 15 mL desalting buffer [KPi (50 mM), NaCl (250 mM), MgCl₂ (1 mM), ThDP (50 µM), pH = 7.4]. 11 mg of freshly purified enzyme ErwE in 5 mL desalting buffer were subsequently added. The enzymatic reaction was stirred in a 25 mL round-bottom flask at room temperature under nitrogen atmosphere. The added amount of 24 was not completely soluble in the reaction mixture. However, after a few hours the insoluble residue of 24 at the bottom of the flask had vanished. Therefore, additional amounts of 24 (twice 80 mg, 0.43 mmol), suspended in 1 mL desalting buffer, were added after 6 (total concentration after addition was 50 mM) and 21 h (total concentration after addition was 63 mM), respectively. After 27 h, the assay was quenched by addition of ethyl acetate. The reaction mixture was extracted three times with 20 mL ethyl acetate and dried over Na₂SO₄. The purification by automated flash column chromatography [Isolera Prime, SNAP Ultra 10 g column, Biotage, Sweden; elution with ethyl acetate/cyclohexane: isocratic 12:88 (1 CV), gradient 10:90 → 100:0 (18 CV), flow rate: 12 mL·min⁻¹] gave 171 mg (628 µmol) of 26. The ¹H NMR spectrum showed 5.7% of minor diastereomer. The yield was 37% (an excess of donor substrate was used).

The purified product was recrystallized from cyclohexane/n-butanol (35:1). Crystals were used for structural elucidation with X-ray crystallography. The absolute configuration was determined as (1R,4R). All crystals, obtained by recrystallisation, were subsequently solved in CDCl₃ and a ¹H NMR spectrum was measured to assure only crystals of one diastereomer were formed. Results are in agreement with the absolute configuration determined by X-ray crystallography.

\[ Rf: 0.30 \text{ (cyclohexane/ethyl acetate = 1:1)} \]

¹H NMR (400 MHz, CDCl₃): \( \delta = 1.14 \text{ ppm (d; } ^2J = 6.3 \text{ Hz; 3H; } CH_3) \), 2.40 (dd; \( ^2J = 17.2 \text{ Hz; } ^3J = 3.2 \text{ Hz; 1H; } CH_xH_y \), 2.55 (dd; \( ^2J = 17.2 \text{ Hz; } ^3J = 8.9 \text{ Hz; 1H; } CH_xH_y \), 4.14–4.23 (m; 1H; CHOHC₃), 5.07 (s; 1H; CHOHCO), AA’BB’ system, centers at \( \delta_A = 7.22 \text{ (2H, } H_{ar} \text{) and } \delta_B = 7.53 \text{ ppm (2H, } H_{ar} \text{)} \).

¹³C NMR (100 MHz, CDCl₃): \( \delta = 22.9 \text{ ppm (CH₃), 46.4 (CH₂), 64.2 (CHOHC₃), 79.8 (CHOHC=O), 123.2 (C₃ar), 129.2 (2C, CH₃), 132.4 (2C, CH₃), 136.7 (CBr₃), 209.2 (C=O)} \).

GC-MS: \( t_R = 12.04 \text{ min, } m/z (%) = 256 [C_{10}H_{13}BrO_3] (1), 244 (4), 228 (4), 187 (72), 185 (100) [C₇H₆BrO], 183 (27), 157 (21), 157 (36), 89 (9) [C₄H₂O₂], 77 (44), 69 (4), 51 (7). \)

CD (CH₃CN): \( \lambda (\Delta \varepsilon_{rel.}) = 300 \text{ nm (–35), 283 (–100), 270 (–69).} \)

C₁₁H₁₃BrO₃: 272.91 g·mol⁻¹
(1R,4R)-1-(4-Bromophenyl)-1,4-dihydroxybutan-2-one (27a)

In a 25 mL round-bottom flask 24 (120 mg, 0.65 mmol, 43 mM) and lyophilized 23 (300 mg) were added to 5 mL water, 5 mL desalting buffer [(KPi (50 mM), NaCl (250 mM), MgCl\(_2\) (1 mM), ThDP (50 µM), pH = 7.4)], and 2 mL DMSO. 4 mg of purified enzyme ErwE in 3 mL desalting buffer were subsequently added. The enzymatic reaction was stirred for 16 h at room temperature under \(N_2\) atmosphere.

The reaction mixture was extracted three times with 7.5 mL ethyl acetate. The organic layers were dried over Na\(_2\)SO\(_4\) and the solvent was evaporated. 4.7 mg (16 µmol, yield = 2.5%) of purified product were obtained from 54.9 mg of crude product by automated flash column chromatography (Isolera Prime, SNAP Ultra 10 g column, Biotage, Sweden; elution with ethyl acetate/cyclohexane: isocratic 5:95 (1 CV), gradient 5:95 \(\rightarrow\) 100:0 (10 CV), flow rate: 12 mL·min\(^{-1}\)).

Considering the chemical shifts and coupling constants of the two geminal protons in \(^1\)H NMR spectra, known from experiments with 26, it is assumed that either (1R,4R) or (1S,4S) product is formed. Results of CD spectroscopy gave evidence that (1R,4R)-27 is formed.

\(R_f\): 0.41 (cyclohexane/ethyl acetate = 1:1)

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta = 0.87\) ppm (t; \(^2J = 7.4\) Hz; 3H; CH\(_3\)), 1.35–1.48 (m, 2H; \(\text{CH}_2\)), 2.38 (dd; \(^2J = 16.9\) Hz, \(^3J = 3.0\) Hz, 1H, C=O\(\text{CH}_2\)), 2.53 (dd; \(^2J = 16.9\) Hz, \(^3J = 9.2\) Hz, 1H, C=O\(\text{CH}_2\)), 3.88–3.95 (m, 1H, \(\text{CH}OH\text{CH}_2\)), 5.08 (s, 1H, \(\text{CHO}\text{H}=\text{O}\)), AA’BB’ system, centers at \(\delta_A = 7.22\) (2H, H\(_{\text{ar}}\)) and \(\delta_B = 7.53\) ppm (2H, H\(_{\text{ar}}\)).

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta = 9.8\) ppm (CH\(_3\)), 29.8 (CH\(_2\)), 44.6 (C=OCH\(_3\)), 69.4 (CHOH), 79.9 (CHOH=O), 123.1 (C\(_{\text{qar}}\)), 129.3 (2C, CH\(_{\text{ar}}\)), 132.4 (2C, CH\(_{\text{ar}}\)), 136.7 (CBr\(_{\text{ar}}\)), 209.4 (C=O).

GC-MS: \(t_R = 11.78\) min; \(m/z\) (%) = 259 (43), 257 (41) [C\(_{10}\)H\(_9\)BrO\(_3\)], 187 (58), 185 (100) [C\(_7\)H\(_5\)BrO], 183 (39), 157 (21), 105 (13), 77 (26), 51 (6).

CD (CH\(_3\)CN): \(\lambda (\Delta\varepsilon_{\text{rel}}) = 300\) nm (–52), 284 (–100), 270 (–62).

C\(_{11}\)H\(_{14}\)O\(_3\): 286.9 g·mol\(^{-1}\)
3-Hydroxy-1-(3-hydroxytetrahydro-2H-pyran-3-yl)-butan-1-one (15)

14 (180 μL), racemic 5 (400 mg), and 1 mL DMSO were resolved in 19 mL desalting buffer [KPi (50 mM), NaCl (250 mM), MgCl₂ (1 mM), ThDP (50 μM), pH = 7.4]. Subsequently, 10 mg of purified enzyme ErwE in 5 mL desalting buffer were added. The reaction mixture was slowly stirred for 21.5 h under N₂ atmosphere at room temperature.

The enzyme was removed with a spin column (cutoff: Mw = 30 kDa), before the solution was extracted three times with 10 mL of ethyl acetate and the organic layers were dried over Na₂SO₄. 32 mg crude product were purified by automated flash column chromatography [Isolera Prime, SNAP Ultra 10 g column, Biotage, Sweden; elution with ethyl acetate/cyclohexane: isocratic 35:65 (2 CV), gradient 35:65 → 100:0 (8 CV), flow rate: 12 mL·min⁻¹]. Fractions containing 15 were identified by TLC; 6.4 mg (34 µmol) of 15 were isolated after purification.

The ¹H NMR spectrum was measured to determine the purity of the product. The whole set of 1D and 2D NMR spectra was measured on the following day, including the repetition of an ¹H NMR spectrum. In the ¹H NMR spectrum, measured on the day after purification, formation of a side product was observed. Some of the signals were related to signals, known from experiments with 5. The side product 41 was identified; it is the product of an elimination of the hydroxy group at C-3′; (E)-41 is formed, concerning coupling constants of the ¹H NMR spectrum. The amount of 41 was approximately 10%. This side product is probably formed from the main product due to slightly acidic conditions in CDCl₃. ¹³C NMR spectra were measured in deuterated acetonitrile, because the signal of one carbon was superimposed by the solvent peak of CDCl₃.

**Rf:** 0.34 (ethyl acetate)

**¹H NMR** (400 MHz, CDCl₃): δ = 1.23 ppm (d; ³J = 6.4 Hz; 3H; CH₃), 1.58–1.66 (m; 1H; C5H₂H₃), 1.70–1.76 (m; 1H; C4H₂H₃), 1.90–1.97 (m; 2H; C5H₂H₂, C4H₂H₂), 2.81–2.84 (m; 2H; C2H₂), 3.45–3.52 (m; 1H; C6H₂H₂), 3.56 (dd; ²J = 11.6 J = 2.3, 1H, C2H₂H₂), 3.64 (d; ²J = 11.6; 1H; C2H₂H₂), 3.90–3.96 (m, 1H, C6H₂H₂), 4.18–4.29 (m; 1H; CHOH).

**¹³C NMR** (100 MHz, CD₃CN): δ = 21.9 ppm (C5), 22.8 (C4′), 31.4 (C4), 46.1 (C2′), 64.2 (C3′), 68.1 (C6), 72.8 (C2), 77.0 (C3), 214.2 (C1′).

**GC-MS:** tᵣ (retro aldol product 42) = 8.83 min; m/z (%): 152 (14), 140 (29), 137 (18), 101 (100), 4 (38), 69 (79), 55 (44). This product has been described in the literature.[¹⁰]

**tᵣ** (23) = 9.59 min; m/z (%): 188 [M]+ (1), 140 (1), 118 (4), 101 (52) [C₅H₉O₂]+, 100 (100), 84 (9), 71 (18), 55 (17).

C₉H₁₆O₄: 188.10 g·mol⁻¹
**MyG**

(R)-1-(4-Bromophenyl)-1,4-dihydroxybutan-2-one (25)

\[ \text{22} \text{CO}_2^– + \text{24} \rightarrow \text{25} \]

24 (60 mg, 0.32 mmol, 26 mM), 22 (91 mg, 0.64 mmol, 52 mM), and 1 mL DMSO were suspended in 9 mL desalting buffer (HEPES (50 mM), NaCl (100 mM), MgCl₂ (2.5 mM), ThDP (50 µM), pH = 7.5). 4.5 mg freshly purified enzyme MygE in 2.5 mL desalting buffer were subsequently added. The enzymatic reaction was stirred at room temperature for 17 h.

The reaction mixture was extracted twice with 10 mL ethyl acetate. The combined organic layers were evaporated and the product was purified by automated flash column chromatography (Isolera Prime, SNAP Ultra 10 g column, Biotage, Sweden; elution with ethyl acetate/cyclohexane: isocratic 10:90 (1 CV), gradient 10:90 → 100:0 (12 CV), flow rate: 12 mL·min⁻¹). The purification gave 1.4 mg (5.4 µmol, yield = 1.7%) of 25.

Formation of 25 was confirmed by comparison of the ¹H NMR spectrum and GC-MS data with the product formed by ErwE. The formation of (R)-25 was determined by CD spectroscopy; the ee was >97%.

CD (CH₃CN): λ (Δεₜₐₜ) = 300 nm (–44), 283 (–100), 270 (–66).

HPLC (Chiralpak AS-H, 25 °C, 0.95 mL·min⁻¹, n-hexane/2-propanol = 92:8): tᵣ = 20.34 min (R); tᵣ = 18.96 min (S); ee >97% (standard assay conditions, 20 h incubation).

(1R,4S)-1-(4-Bromophenyl)-1,4-dihydropentan-2-one (26b)

\[ \text{5} \text{CO}_2^– + \text{24} \rightarrow \text{26b} \]

24 (10 mg, 0.05 mmol, 36 mM), racemic 5 (20 mg), and 100 µL DMSO were suspended in 900 µL desalting buffer [HEPES (50 mM), NaCl (100 mM), MgCl₂ (2.5 mM), ThDP (50 µM), pH = 7.5]. Subsequently, 1 mg freshly purified enzyme MygE in 500 µL desalting buffer was added. Two assays with the above mentioned composition were incubated at 30 °C and 300 rpm for 20 h.

Both assays were extracted three times with 300 µL ethyl acetate. The organic phases were combined and evaporated; formation of 26 was confirmed by GC-MS (comparison with data of 26, formed by ErwE). The diastereomeric ratio (81:19) was determined by ¹H NMR spectroscopy prior to purification. The raw product was purified by automated flash column chromatography [Isolera Prime, SNAP Ultra 10 g column, Biotage, Sweden; elution with ethyl acetate/cyclohexane: isocratic 10:90 (1 CV), gradient 10:90 → 100:0 (12 CV), flow rate: 12 mL·min⁻¹]. Isolated 26 was still contaminated with impurities after the purification.

Chemical shifts and coupling constants of the geminal protons which are known for (1R,4R)-26, were compared. Data show that ErwE and MyGE form different diastereomers of 26. The results of CD spectroscopy reveal that MyGE forms (1R,4S)-26.

¹H NMR (400 MHz, CDCl₃): δ = 1.15 ppm (d; ²J = 6.4 Hz; 3H; CH₃), 2.44 (dd; ²J = 17.4 Hz, ³J = 8.4 Hz; 1H; CH₂H₃), 2.56 (dd; ²J = 17.4 Hz, ³J = 3.4 Hz; 1H; CH₂H₃), 4.17–4.26 (m; 1H; CHOCH₃), 5.08 (s; 1H; CHOHC=O), AA’BB’ system, centers at δₜ = 7.21 (2H, Hₐ) and δₜ = 7.53 ppm (2H, Hₐ).
$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 22.7$ ppm (CH$_3$), 46.2 (CH$_2$), 64.0 (CHOHCH$_3$), 79.4 (CHOHCO), 123.1 (C$_{q}$ar), 129.1 (2C, CHar), 132.4 (2C, CHar), 136.6 (CBr$_{ar}$), 209.4 (C=O).

CD (CH$_3$CN): $\lambda (\Delta\varepsilon_{rel}) = 300$ nm (–42), 282 (–100), (–52).

C$_{11}$H$_{13}$BrO$_3$: 272.91 g·mol$^{-1}$

(1$R,4S$)-1-(4-Bromophenyl)-1,4-dihydroxybutan-2-one (27b)

24 (5.6 mg, 0.3 mmol, 20 mM), 23 (23 mg), and 100 µL DMSO were mixed in 900 µL desalting buffer [HEPES (50 mM), NaCl (100 mM), MgCl$_2$ (2.5 mM), ThDP (50 µM), pH = 7.5]. Subsequently, 1 mg freshly purified enzyme MygE in 500 µL desalting buffer was added and incubated at 30 °C and 300 rpm for 21 h.

The assay was extracted three times with 300 µL ethyl acetate. The diastereomeric ratio was determined from the $^1$H NMR spectrum prior to purification; 37% of the minor diastereomer was formed. After evaporation the raw product was partly purified by manual flash column chromatography (silica gel 60, particle size 40–63 µm, Merck). 24 was eluted from the column with ethyl acetate/cyclohexane 10:90; the elution of 24 was detected on TLC. Ethyl acetate was subsequently used to elute 27. The partly purified product was identified by GC-MS (comparison with data of 27a formed by ErwE).

Chemical shifts and coupling constants of geminal protons, known for (1$R,4R$)-27 formed by ErwE, were compared; results showed that ErwE and MyGE form different diastereomers of 27. The results of CD spectroscopy reveal that MyGE forms (1$R,4S$)-27.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta = 0.89$ ppm (t; $^3J = 7.5$ Hz; 3H; CH$_3$), 1.38–1.50 (m, 2H; CH$_2$CH$_3$), 2.44 (dd; $^2J = 17.2$ Hz, $^3J = 8.7$ Hz, 1H, C=OCH$_2$H$_3$), 2.56 (dd; $^2J = 17.2$ Hz, $^3J = 3.2$ Hz, 1H, C=OCH$_2$H$_3$), 3.88–4.00 (m, 1H, CHOCH$_2$CH$_3$), 5.08 (s, 1H, CHOHC=O), AA'BB' system, centers at $\delta_A = 7.21$ (2H, H$_{ar}$) and $\delta_B = 7.53$ ppm (2H, H$_{ar}$).

CD (CH$_3$CN): $\lambda (\Delta\varepsilon_{rel}) = 300$ nm (–49), 285 (–100), 270 (–63).
(R)-1-(4-Bromophenyl)-1,4-dihydroxybutan-2-one (25)

24 (37 mg, 0.2 mmol, 20 mM) and 22 (50 mg, 0.35 mmol, 35 mM) were mixed with 1 mL DMSO and 4 mL desalting buffer [HEPES (50 mM), NaCl (100 mM), MgCl₂ (2.5 mM), ThDP (50 µM), pH = 7.5]. After addition of 5 mL cell suspension (MMAR_2232) the enzymatic reaction was stirred at room temperature for 25 h. The reaction mixture was sonicated twice for 2 min. Cell debris was sedimented (12,600 × g, 4 °C, 20 min) and the supernatant was extracted twice with 10 mL ethyl acetate.

Formation of 25 was confirmed by comparison of the ¹H NMR spectrum and GC-MS data with the product formed by ErwE. The formation of (R)-product was identified with CD spectroscopy, the ee was >65%.

CD (CH₃CN): λ (Δεₑₑₑ) = 300 nm (–45), 286 (–100), 270 (–66).

HPLC (Chiralpak AS-H, 25 °C, 1 mL·min⁻¹, n-hexane/2-propanol = 92:8): tᵣ = 20.3 min (R); tᵣ = 19.0 min (S); ee (MyGE) >65%.

(1R,4R)-1-(4-Bromophenyl)-1,4-dihydroxypentan-2-one (26a)

24 (37 mg, 0.02 mmol, 20 mM) and racemic 5 (150 mg) were resuspended in 1 mL DMSO and 2 mL desalting buffer (HEPES (50 mM), NaCl (100 mM), MgCl₂ (2.5 mM), ThDP (50 µM), pH = 7.5). 7 mL cell suspension (MMAR_2232) was added and the enzymatic reaction was stirred at room temperature for 19 h. The reaction mixture was sonicated twice for 2 min. It was subsequently centrifuged (12,600 × g, 4 °C, 20 min) and the supernatant was extracted twice with 8 mL ethyl acetate. The combined organic layers were evaporated and the raw product was purified with automated flash column chromatography (Isolera Prime, SNAP Ultra 10 g column, Biotage, Sweden; elution with ethyl acetate/cyclohexane: isocratic 10:90 (1 CV), gradient 10:90 → 100:0 (12 CV), flow rate: 12 mL·min⁻¹). Formation of 26 was confirmed by comparison of the ¹H NMR spectrum with the product of ErwE; the same diastereomer of 26 is formed with MMAR_2332 and ErwE. The diastereomeric ratio (78:22) of 26 was determined by ¹H NMR spectroscopy before purification.

The raw product before purification was a mixture of related products. In addition to 26, substantial amounts of 4-bromobenzyl alcohol were detected in the ¹H NMR spectrum (ratio was 1:3). A complete separation of 26 was not achieved by automated flash column chromatography. 4-bromobenzyl alcohol is probably the reduction product of 24, catalyzed by unspecific host reductases of the expression organism.
4. Analytical scale assay:

To determine and compare the conversion for different substrates with both ErwE and MyGE a standard protocol was used. The products of those conversions were also used for full characterisation, if suitable, otherwise a preparative scale assay, followed by a purification step, was performed.

Benzaldehyde (20 mM) was used as acceptor substrate, because it is fully dissolvable in buffer containing 100 µL DMSO, a requirement for the calculation of conversion from extracted assays. The donor substrate was used in a concentration of 50 mM and dissolved in buffer. For HOPA (5) and HOHA (23) no molarity was determinable. 20 mg of HOPA and 23 mg of HOHA were added as donor substrate. Most donor substrates were used as sodium salts of the corresponding α-ketoacids. If the α-ketoacid itself was applied, the pH was adjusted to ~ 7.5 by addition of sodium hydroxid (5 M).

The assays had a total volume of 1.5 mL. They were composed of desalting buffer and 100 µL of DMSO. A total amount of 1.5 mg freshly purified enzyme in desalting buffer was added to the assay. Those assays were incubated at 30 °C and 300 rpm for 20 hours.

1 mL of assay was extracted with 650 µL CDCl3 (30 seconds vortexing and 3 min centrifugation at 14 000 rpm). Conversion was calculated by integration of the signal of the aldehyde proton of benzaldehyde compared to the signal of the carbinol proton (product) in 1H NMR spectra.

Assays with HOBA and 4-bromobenzaldehyde were performed under equal conditions for the determination of the enantiomeric excess.

Analytical data of products

(R)-1-Hydroxy-1-phenylpropan-2-one [(R)-30]

(R)-1-Hydroxy-1-phenylpropan-2-one [(R)-30] was identified by comparison of the 1H NMR spectrum with literature data.[11]

HPLC (Chiralcel OD-H, 25 °C, 0.5 mL-min⁻¹, n-hexane/2-propanol = 95:5): t_R = 24.6 min (R); t_S = 21.6 min (S); ee (MyGE) >98%.

Conversion: MyGE: 47%, 37%, 35%.

C₉H₁₀O₂: 150.07 g·mol⁻¹
(R)-1-Hydroxy-1-phenylbutan-2-one (31)

\[
\begin{align*}
\text{R}-1-\text{Hydroxy-1-phenylbutan-2-one (31)}
\end{align*}
\]

(R)-1-Hydroxy-1-phenylbutan-2-one (31) was identified by comparison of the \(^1\)H NMR spectrum with literature data.\(^{[12]}\)

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta = 1.00\) ppm (s; 3H; CH\(_3\)), 2.32 (dd; \(^2\)J = 17.8 Hz, \(^3\)J = 7.3 Hz; 1H; CH\(_2\)H\(_3\)), 2.39 (dd; \(^2\)J = 17.8 Hz, \(^3\)J = 7.3 Hz; 1H; CH\(_2\)H\(_3\)), 5.08 (s; 1H; CHO), 7.28–7.40 (m; 5H; CH\(_\text{ar}\)).

HPLC (Chiral OM, 40 °C, 0.5 mL·min\(^{-1}\), n-hexane/2-propanol = 95:5): \(t_R = 26.1\) min (R); \(t_R = 22.3\) min (S); ee (MyGE) >90%.

Conversion: MyGE: 30%, 42%, 33%.

C\(_{10}\)H\(_{12}\)O\(_2\): 164.08 g·mol\(^{-1}\)

(R)-1-Hydroxy-1-phenylpentan-2-one (32)

\[
\begin{align*}
\text{R}-1-\text{Hydroxy-1-phenylpentan-2-one (32)}
\end{align*}
\]

(R)-1-Hydroxy-1-phenylpentan-2-one (32) was identified by comparison of the \(^1\)H NMR spectrum with literature data.\(^{[12]}\)

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta = 0.80\) ppm (t; \(^3\)J = 7.4 Hz; 3H; CH\(_3\)), 1.44–1.64 (m; 2H; CH\(_2\)CH\(_3\)), 2.27 (ddd; \(^2\)J = 17.7 Hz, \(^3\)J = 8.0 Hz; 1H; COCH\(_2\)H\(_3\)), 2.36 (ddd; \(^2\)J = 17.7 Hz, \(^3\)J = 8.1 Hz, \(^3\)J = 6.5 Hz; 1H; COCH\(_2\)H\(_3\)), 5.08 (s; 1H; CHO), 7.29–7.41 (m; 5H; CH\(_\text{ar}\)).

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta = 13.7\) ppm (CH\(_3\)), 17.3 (CH\(_2\)CH\(_3\)), 39.9 (CH\(_2\)CH\(_2\)), 80.1 (CHOH), 127.6 (2C, CH\(_\text{ar}\)), 129.0 (CH\(_\text{ar}\)), 129.2 (2C, CH\(_\text{ar}\)), 137.7 (C\(_\text{ar}\)), 208.0 (C=O).

GC-MS: \(t_R = 9.32\) min; \(m/z\) (%): 178 (3) [M]\(^+\), 107 (100) [C\(_7\)H\(_7\)O]\(^+\), 79 (82), 71 (11) [C\(_4\)H\(_7\)O]\(^+\), 51 (10).

CD (CH\(_3\)CN): \(\lambda (\Delta\varepsilon) = 300\) nm (−43), 283 (−120.8), 270 (−67) (MyGE).

Conversion: MyGE: 86%, 84%, 86%.

C\(_{11}\)H\(_{14}\)O\(_2\): 178.10 g·mol\(^{-1}\)
(R)-1-Hydroxy-4-methyl-1-phenylpentan-2-one (33)

(R)-1-Hydroxy-4-methyl-1-phenylpentan-2-one (33) was identified by comparison of the $^1$H NMR spectrum with literature data.\textsuperscript{[12]}

$^1$H NMR (400 MHz, CDCl$_3$): $\delta = 0.74$ ppm (d; $^3$J = 6.5 Hz; 3H; CH$_3$), 0.88 (d; $^3$J = 6.6 Hz; 3H; CH$_3$), 2.10 (sept; $^3$J = 6.6 Hz, 1H; CH(CH$_3$)$_2$), 2.17 (dd; $^2$J = 16.2, Hz, $^3$J = 7.0 Hz; 1H; CH$_3$H$_2$), 2.27 (dd; $^2$J = 16.2, Hz, $^3$J = 6.8 Hz; 1H; CH$_3$H$_2$), 5.04 (s; 1H; CH$_2$OH), 7.28–7.41 (m; 5H; CH$_2$Ar).

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 22.4$ ppm (CH$_3$), 22.6 (CH$_3$), 24.7 (CH), 46.8 (CH$_2$), 80.2 (CHOH), 127.6 (2C, CH$_2$), 128.8 (CH$_2$), 129.1 (2C, CH$_2$), 138.0 (C$_q$Ar), 209.2 (C=O).

GC-MS: $t_R = 9.60$ min; m/z (%) = 192 (1) [M]$^+$, 107 (100) [C$_7$H$_7$O]$^+$, 85 (13) [C$_5$H$_7$O]$^+$, 79 (29), 57 (13) [C$_4$H$_9$]+, 51 (4).

CD (CH$_3$CN): $\lambda$ ($\Delta\varepsilon_{\text{rel.}}$) = 300 nm (–54), 284 (–100), 270 (–64) (MyGE).

Conversion: MyGE: 16%, 26%, 23%.

C$_{12}$H$_{16}$O$_2$: 192.12 g·mol$^{-1}$

(R)-1-Hydroxy-4-(methylthio)-1-phenylbutan-2-one (34)

(R)-1-Hydroxy-4-(methylthio)-1-phenylbutan-2-one (34) was identified by comparison of the $^1$H NMR spectrum with literature data.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta = 1.98$ ppm (s; 3H; CH$_3$), 2.55–2.75 (m; 4H; 2 x CH$_2$), 5.12 (s; 1H; CH$_2$OH), 7.30–7.42 (m; 5H; CH$_2$Ar).

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 15.8$ ppm (CH$_3$), 28.0, 37.8 (CH$_2$), 80.1 (CHOH), 127.6 (2C, CH$_2$), 129.0 (CH$_2$), 129.2 (2C, CH$_2$), 137.7 (C$_q$Ar), 208.0 (C=O).

GC-MS: $t_R = 11.39$ min; m/z (%) = 210 (8) [C$_{11}$H$_{14}$O$_2$S]$^+$, 154 (4), 149 (2) [C$_9$H$_7$O$_2$]$^+$, 136 (26), 107 (100) [C$_7$H$_7$O]$^+$, 79 (69), 61 (25) [C$_2$H$_5$S]$^+$, 51 (11).

CD (CH$_3$CN): $\lambda$ ($\Delta\varepsilon_{\text{rel.}}$) = 300 nm (–67), 287 (–100), 270 (–68) (ErwE).

CD (CH$_3$CN): $\lambda$ ($\Delta\varepsilon_{\text{rel.}}$) = 300 nm (–48), 284 (–100), 270 (–64) (MyGE).

Conversion: ErwE: 10%, 108%.

MyGE: 73%, 79%, 79%.

C$_{11}$H$_{14}$O$_2$S: 210.07 g·mol$^{-1}$
(R)-1-Hydroxy-1-phenyloctan-2-one (35)

\[ \text{35} \]

**\(^1\)H NMR** (400 MHz, CDCl\(_3\)): \( \delta = \) ppm 0.83 (t; \( \overset{2}{J} = 7.1 \) Hz; 3H; CH\(_3\)), 1.09–1.29 (m; 6H; 3 x CH\(_2\)), 1.43–1.56 (m; 2H; COCH\(_2\)CH\(_3\)), 2.29 (ddd; \( \overset{2}{J} = 16.9 \) Hz, \( \overset{3}{J} = 8.1 \) Hz, \( \overset{3}{J} = 6.8 \) Hz; 1H; COCH\(_2\)H\(_2\)), 2.37 (ddd; \( \overset{2}{J} = 16.9 \) Hz, \( \overset{3}{J} = 8.3 \) Hz, \( \overset{3}{J} = 6.4 \) Hz; 1H; COCH\(_2\)H\(_2\)), 5.08 (s; 1H; CHO\(_{\alpha}\)), 7.28–7.41 (m; 5H; CH\(_{\alpha\alpha}\)).

**\(^13\)C NMR** (100 MHz, CDCl\(_3\)): \( \delta = \) ppm 14.1 (CH\(_3\)), 22.5, 23.8, 28.8, 31.5, 38.0 (CH\(_2\)), 79.8 (CHOH), 127.6 (2C, CH\(_{\alpha\alpha}\)), 128.8 (CH\(_{\alpha\alpha}\)), 128.9 (100 MHz, CDCl\(_3\)), 138.1 (C\(_\alpha\)), 209.9 (C=O).

**GC-MS:** \( t_{fr} = 11.19 \) min; \( m/z (\%) = 220 (1) [M]^+, 191 (0.2) [C\(_{12}\)H\(_{15}\)O\(_2\)]^+, 113 (13) [C\(_{2}\)H\(_{13}\)O\(_2\)]^+, 107 (100) [C\(_7\)H\(_7\)O\(_2\)]^+, 85 (5) [C\(_6\)H\(_{13}\)]^+, 79 (23), 55 (5).

**CD:** (CH3CN) \( \lambda \) (rel \( \Delta \varepsilon \)) [nm] = 300 (–43), 282 (–100), 270 (–68) (MyGE).

**Conversion:** MyGE: 27.0%, 23%, 22%

C\(_{14}\)H\(_{20}\)O\(_2\): 220.15 g·mol\(^{-1}\)

(\(R\))-1,4-Dihydroxy-1-phenylbutan-2-one (36)

\[ \text{36} \]

**\(^1\)H NMR** (400 MHz, CDCl\(_3\)): \( \delta = \) ppm 2.50 ppm (ddd; \( \overset{2}{J} = 17.2 \) Hz, \( \overset{3}{J} = 6.4 \) Hz, \( \overset{3}{J} = 4.8 \) Hz; 1H; CH\(_3\)), 2.63 (ddd; \( \overset{2}{J} = 17.2 \) Hz, \( \overset{3}{J} = 7.2 \) Hz, \( \overset{3}{J} = 4.8 \) Hz; 1H; CH\(_3\)), 3.68–3.80 (m; 2H; CH\(_2\)), 5.08 (s; 1H; CHO\(_{\alpha}\)), 7.23–7.33 (m; 5H; CH\(_{\alpha\alpha}\)).

**\(^13\)C NMR** (100 MHz, CDCl\(_3\)): \( \delta = \) ppm 40.4 ppm (CH\(_2\)), 57.5 (CH\(_2\)OH), 80.0 (CHOH), 127.3 (2C, CH\(_{\alpha\alpha}\)), 128.6 (CH\(_{\alpha\alpha}\)), 128.9 (2C, CH\(_{\alpha\alpha}\)), 137.9 (C\(_\alpha\)), 209.6 (C=O).

**GC-MS:** \( t_{fr} = 10.40 \) min; \( m/z (\%) = 180 (0.1), 152 (21), 134 (4) 107 (100) [C\(_7\)H\(_7\)O\(_2\)]^+, 105 (28), 79 (57), 73 (2), 105 (5) 51 (1).

**CD** (CH\(_3\)CN): \( \lambda \) (rel \( \Delta \varepsilon \)) = 300 nm (–44), 283 (–100), 270 (–67) (ErwE).

(\(R\))-Dihydroxy-1-phenylbutan-2-one (36)

**Conversion:** ErwE: 37%, 29%, 99%.

**MyGE:** 19%, 17%, 18%.

C\(_{10}\)H\(_{12}\)O\(_2\): 180.08 g·mol\(^{-1}\)
(1R,4R)-1,4-Dihydroxy-1-phenylpentan-2-one [(1R,4R)-37]

NMR data are given for (1R,4R)-1,4-dihydroxy-1-phenylpentan-2-one [(1R,4R)-37], synthesized with ErwE. MyGE gave (1R,4S)-1,4-dihydroxy-1-phenylpentan-2-one [(1R,4S)-37].

^1H NMR (400 MHz, CDCl₃): δ = 1.11 ppm (d; ^3J = 6.3 Hz; 3H; CH₃), 2.42 (dd; ^2J = 17.3 Hz, ^3J = 3.2 Hz; 1H; CH₃H), 2.55 (dd; ^2J = 17.3 Hz, ^3J = 8.9 Hz; 1H; CH₃H), 4.17 (m; 1H; CHOCH₂), 5.11 (s; 1H; CHOHCO), 7.30–7.43 (m; 5H; CH₃ara).

^13C NMR (100 MHz, CDCl₃): δ = 22.6 ppm (CH₃), 46.2 (CH₂), 64.0 (CHOCH₂), 80.2 (CHOHCO), 127.4 (2C, CHara), 128.9 (CHara), 129.1 (2C, CHara), 137.5 (Cₚₐ₉), 209.8 (C=O).

GC-MS: tR = 10.44 min; m/z (%): 194 (1) [M]^+, 166 (18), 148 (34), 107 (100) [C₇H₇O]^+, 105 (74), 79 (100) [C₆H₇]^+, 77 (90) [C₆H₅]^+, 51 (15).

CD (CH₃CN): λ (Δεrel.) = 300 nm (–52), 284 (–100), 270 (–63) (ErwE).

Conversion: ErwE: 50%, 50%, 46%.

C₁₁H₁₄O₃: 194.09 g·mol⁻¹

(1R,4S)-1,4-Dihydroxy-1-phenylpentan-2-one [(1R,4S)-37]

CD (CH₃CN): λ (Δεrel.) = 300 nm (–52), 283 (–100), 270 (–65) (MyGE).

Conversion: MyGE: 1%, 1%, 0%.

C₁₁H₁₄O₃: 194.09 g·mol⁻¹
\((1R,4R)\)-1,4-Dihydroxy-1-phenylhexan-2-one [(1\(R\),\(R\))-38]

\[
\begin{align*}
\text{OH} & \quad \text{O} \\
\text{OH} & \quad \text{CH} \quad \text{CH} \\
\text{O} & \quad \text{H} \\
\end{align*}
\]

NMR data are given for (1\(R\),\(R\))-1,4-dihydroxy-1-phenylhexan-2-one [(1\(R\),\(R\))-38], synthesized with ErwE. MyGE gave (1\(R\),\(S\))-1,4-dihydroxy-1-phenylhexan-2-one [(1\(R\),\(S\))-38], \(\Delta\varepsilon_{\text{rel.}}\) of (1\(R\),\(S\))-38 is lower than that of (1\(R\),\(R\))-38.

\(1\text{H NMR}\) (400 MHz, CDCl\(_3\)): \(\delta = 0.85\) ppm (t, \(^3J = 7.4\) Hz, 3H; CH\(_3\)), 1.35–1.45 (m; 2H; CH\(_2\)CH\(_3\)), 2.42 (dd; \(^2J = 17.1\) Hz, \(^3J = 3.0\) Hz; 1H; CH\(_2\)CH\(_3\)OH), 2.55 (dd; \(^2J = 17.0\) Hz, \(^3J = 9.0\) Hz; 1H; CH\(_2\)CH\(_3\)COH), 3.86–3.92 (m; 1H; CH\(_2\)OH), 5.12 (s; 1H; CH\(_2\)OHC), 7.31–7.43 (m; 5H; CH\(_{\text{ar}}\)).

\(13\text{C NMR}\) (100 MHz, CDCl\(_3\)): \(\delta = 9.8\) ppm (CH\(_3\)), 29.7 (CH\(_2\)CH\(_3\)), 44.6 (CH\(_2\)CHOH), 69.4 (CHOHCH\(_2\)), 80.6 (CHOHCOH), 127.6 (2C, CH\(_{\text{ar}}\)), 128.9 (CH\(_{\text{ar}}\)), 129.1 (2C, CH\(_{\text{ar}}\)), 137.7 (C\(_{\text{ar}}\)), 210.2 (C=O).

\(\text{GC-MS}\): \(t_R = 11.00\) min; \(m/z\) (%) = 207 (4), 180 (5), 162 (9), 133 (3), 107 (100) [C\(_7\)H\(_4\)O], 105 (38) [C\(_7\)H\(_5\)O], 79 (40), 77 (36) [C\(_6\)H\(_5\)], 51 (8).

\(\text{CD} (\text{CH}_3\text{CN})\): \(\lambda (\Delta\varepsilon_{\text{rel.}}) = 300\) nm (–46), 283 (–100), 270 (–66) (ErwE).

**Conversion:** ErwE: 83%, 85%, 61%.

\(\text{C}_{12}\text{H}_{16}\text{O}_3\): 208.11 g\cdot\text{mol}^{-1}

\((1R,4S)\)-1,4-Dihydroxy-1-phenylhexan-2-one [(1\(R\),\(S\))-38]

\[
\begin{align*}
\text{OH} & \quad \text{O} \\
\text{OH} & \quad \text{CH} \quad \text{CH} \\
\text{O} & \quad \text{H} \\
\end{align*}
\]

\(\text{CD} (\text{CH}_3\text{CN})\): \(\lambda (\Delta\varepsilon_{\text{rel.}}) = 300\) nm (–46), 283 (–100), 270 (–66) (MyGE).

**Conversion:** MyGE: 2%, 2%, 1%.

\(\text{C}_{12}\text{H}_{16}\text{O}_3\): 208.11 g\cdot\text{mol}^{-1}
5. Crystallography data

X-ray Crystallography

X-ray crystallographic data were collected on a micro-source equipped Bruker AXS diffractometer. Crystal samples were handled under immersion oil and were loop-mounted in a cold nitrogen stream. The crystals were kept at 100 K during data collection. Both compounds crystallize in the monoclinic space group \( P2_1 \). Their crystal structures were solved using Direct Methods as implemented in the SHELXS-2013\[^{[13]}\] program. The structure refinements were performed with SHELXL-2013\[^{[13]}\], where all non-hydrogen atoms were refined with anisotropic displacement parameters. The crystal data of both compounds are collected in the Tables 1 and 2.\[^{[a]}\] Structure drawings and the analysis of the hydrogen bonding systems were performed with the program DrawXTL.\[^{[14]}\]

\[^{[a]}\] Crystallographic data (including structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre, CCDC, 12 Union Road, Cambridge CB21EZ, UK. Copies of the data can be obtained free of charge on quoting the depository numbers CCDC-2110665 (25) and CCDC-2110666 (26a). (Fax: +44-1223-336-033; E-Mail: deposit@ccdc.cam.ac.uk, http://www.ccdc.cam.ac.uk).
Table 1: Crystal data of the structures of 25 (left) and 26a (right).

|                        | 25                  | 26a                  |
|------------------------|---------------------|----------------------|
| Empirical formula      | C₁₀H₁₁BrO₃          | C₁₁H₁₃BrO₃           |
| Formular weight        | 259.10              | 273.12               |
| Crystal system         | Monoclinic          |                      |
| Space group, no.       | P 2₁, no. 4         |                      |
| Temperature, K         | 100                 |                      |
| Lattice parameters, a  | 759.530(10)         | 542.70(4)            |
| pm , °                 | 565.540(10)         | 1046.70(7)           |
| b                      | 1163.80(2)          | 1005.38(7)           |
| c                      | 97.6911(7)          | 96.118(4)            |
| β                      | 495.407(14)         | 567.85(7)            |
| Volume of the unit cell, 10⁶ pm³ |                      |                      |
| Z                      | 2                   |                      |
| Density (Xray), gcm⁻³ ]| 1.737               | 1.597                |
| Diffractometer, radiation | Bruker AXS CCD, Mo-Kα |                      |
| Absorption coefficient | 4.126               | 3.604                |
| μMo-Kα, mm⁻¹           | 1.8-32.5            | 2.0-27.5             |
| θ range, °             | 19765               | 6688                 |
| Reflections collected  | 3541                | 2438                 |
| Independent reflections| 0.0257              | 0.0259               |
| Refinement             | Lorentz, polarisation, absorption (Multi-Scan™) |
| Structure determination| SHELXL-2013[15]      |                      |
| Refinement             | SHELXL-2013[15]      |                      |
| Number of free parameters| 135                 | 140                  |
| Goodness-of-fit on F²  | 1.055               | 1.073                |
| Flack-x parameter      | 0.029(3)            | 0.035(5)             |
| R values [for refl. with I ≥ 2σ(I)] | R₁      | 0.0189               | 0.0228               |
|                         | wR2                 | 0.0477               | 0.0585               |
| R values (all data)    | R₁                  | 0.0202               | 0.0243               |
|                         | wR2                 | 0.0481               | 0.0590               |
| Residual elect. density, e⁻¹0⁻⁶ pm⁻³ | +0.51/-0.22         | +1.01/-0.46          |
Table 2: Atomic coordinates and equivalent isotropic displacement parameters/pm\(^2\) for the crystal structures of 25 (top) and 26a (bottom). All atoms at general Wykooff position.

| Atom | x          | y          | z          | U\(_{\text{equiv.}}\) |
|------|------------|------------|------------|----------------------|
| 25   |            |            |            |                      |
| Br   | 0.35155(2) | 0.43966(5) | 0.64881(2) | 213.1(6)             |
| C(1) | 0.0779(2)  | 0.1561(3)  | 0.14219(14)| 118(3)               |
| C(2) | -0.1247(2) | 0.1837(3)  | 0.12320(14)| 117(3)               |
| C(3) | -0.1948(2) | 0.4210(5)  | 0.15638(12)| 136(3)               |
| C(4) | 0.39096(19)| 0.4563(5)  | 0.11437(13)| 150(3)               |
| C(6) | 0.1503(2)  | 0.2237(3)  | 0.26623(14)| 116(3)               |
| C(7) | 0.1265(2)  | 0.0696(4)  | 0.35693(14)| 151(3)               |
| C(8) | 0.1864(2)  | 0.1320(4)  | 0.47137(15)| 171(3)               |
| C(9) | 0.2706(2)  | 0.3476(4)  | 0.49362(14)| 150(3)               |
| C(10)| 0.2977(2)  | 0.5021(3)  | 0.40531(15)| 167(3)               |
| C(11)| 0.23612(18)| 0.4386(5)  | 0.29076(12)| 143(2)               |
| O(1) | 0.12074(15)| -0.0774(3) | 0.11500(10)| 152(2)               |
| O(2) | 0.22030(16)| 0.0220(3)  | 0.08530(11)| 157(2)               |
| O(4) | 0.41614(15)| 0.4588(3)  | -0.00940(9)| 153(2)               |
| 26a  |            |            |            |                      |
| Br   | 0.01095(5) | 0.90924(3) | 0.46599(3) | 235.6(11)            |
| C(1) | 0.0798(6)  | 0.4928(3)  | 0.0340(3)  | 146(6)               |
| C(2) | 0.3062(5)  | 0.4107(4)  | 0.0754(2)  | 143(5)               |
| C(3) | 0.3076(6)  | 0.3403(3)  | 0.2070(3)  | 160(6)               |
| C(4) | 0.4808(6)  | 0.2257(3)  | 0.2206(3)  | 166(6)               |
| C(5) | 0.5028(7)  | 0.1704(3)  | 0.3598(4)  | 249(8)               |
| C(6) | 0.0590(5)  | 0.5952(3)  | 0.1396(3)  | 139(6)               |
| C(7) | 0.2266(6)  | 0.6974(3)  | 0.1503(3)  | 173(6)               |
| C(8) | 0.2130(6)  | 0.7904(3)  | 0.2465(3)  | 195(7)               |
| C(9) | 0.0307(6)  | 0.7812(3)  | 0.3328(3)  | 176(6)               |
| C(10)| -0.1367(6)| 0.6821(3)  | 0.3247(3)  | 189(7)               |
| C(11)| -0.1203(6)| 0.5892(3)  | 0.2278(3)  | 172(6)               |
| O(1) | 0.1039(4)  | 0.5450(2)  | -0.0932(2) | 182(5)               |
| O(2) | 0.4796(3)  | 0.4078(4)  | 0.0086(2)  | 186(4)               |
| O(4) | 0.3790(4)  | 0.1335(2)  | 0.1249(2)  | 188(5)               |
Figure 1: Thermal ellipsoid plot (90% level) of the molecules 25 (top) and 26a (bottom). Selected bond distances [Å] for 25: Br-C9 1.901(2), C1-O1 1.406(3), C1-C6 1.523(2), C1-C2 1.533(2), C2-O2 1.213(2), C2-C3 1.513(3), C3-C4 1.517(2), C4-O4 1.4276(18), C6-C11 1.391(3), C6-C7 1.399(2), C7-C8 1.394(2), C8-C9 1.385(3), C9-C10 1.385(3), C10-C11 1.399(2) and for 26a: Br-C9 1.906(3), C1-O1 1.409(4), C1-C2 1.521(4), C1-C6 1.522(4), C2-O2 1.213(3), C2-C3 1.514(4), C3-C4 1.522(4), C4-O4 1.431(4), C4-C5 1.507(5), C6-C11 1.386(5), C6-C7 1.400(4), C7-C8 1.380(4), C8-C9 1.387(5), C9-C10 1.375(4), C10-C11 1.386(4).
6. NMR spectra:

\[ ^1H \text{ NMR:} \]

![NMR spectra images]
$^{13}$C NMR:

25

26a
(1R,4R)-37

(1R,4R)-38
7. Nucleotide and amino acid sequences of ErwE and MyGE

**ErwE** – nucleotide sequence from *Erwinia carotovora* subsp. *atroseptica* SCR1043

```
ATACGAGTGGCAGATTTTTATTGTTCAGCACCTTCGTAATATCGGTGTTGACACCGTATTTCTATTGAACGGTGGGGG
CATGATGCATATGGTTGATGCCCTGGCTCGATGTGAAGGTATGCACTATGTTTGCAGCCACCATGAGCAGGCATCT
GCTATTGCTGCTGCTGATGCGTATGCTCGTCTTTCTGGTAAATTAGGCGTCTGCTACGCAACCGCAGGGCCGGGTGCAA
CCAACATCCTTACCGGGGTGCTAGTGGTGGGCAAAGATAGTTCTCCTGCGCTTTATTACGGGCCAAAGTAAAAGT
ACACAAACCATTGCGATGTCTGGCATAAAAGGATTGCGTCAGTTTGGTACATTTGAAGTGAATACCGTACCCATCAT
GGAAAGCGTGACTAAATATAGCCACATGCTGACTGATGCGAGCACGATTAAATATCATCTGGAAAAAGCAATACGG
AAGCGACATCGGGTCGACCTGGGCCTGTTTTACTTGATATTC
```

**ErwE** – nucleotide sequence (codon optimized)

```
GACGACGACAAGCATATGC
```

**ErwE** – amino acid sequence

```
MGHHHHHHHHHHSSHGHIDDDDKHRMVDVFIVOHLRNGIVDTFLVNLNgNGMGMMDVALRCEGMHVGCHHEQ
ASIAADADAVRLSKGLKCVATAGPATNTLTGVGAVGQDSSPALFTGQSKSTQTMSIKGLRQFGETVNTV
PIMESVSKSHTMDASTKLYHLEAIETHSGPRVPIVPLLDPIDQLGILPEGDFEEPTALPESNIOQIA
VSAJKRIVLPILVGLGRLVTSFEAKLKLFLWYKPVVQMTGCQVDKLVGMYMEHPFELWQHSPGPDRQNFAVOTADVIL
SLGTSLSQTTGNLWAPPPLEFQALSLALAVTDEQTLRGRVSRKVTLPRLKDRGAVK
```

**ErwE** – amino acid sequence (codon optimized)

```
MGHHHHHHHHHHSSHGHIDDDDKHRMVDVFIVOHLRNGIVDTFLVNLNgNGMGMMDVALRCEGMHVGCHHEQ
```

EkiemSaAiled
**myGE – nucleotide sequence from *Mycobacterium gastri* ‘wayne’**

GTGCAGCCGCGCCGACAGCGATGAGTTGCTGGTCAACCGGATGCGCGAGGCCGGGAT
ATCCCTGCTGTGCGGGCTGCCGAC
CTCGCGGCTCGACACGTTGCTGGCCCGGCTCGCCCGCGACGACGACTTCGACATCGTGCTGACCC
GGCACGAAG
GCGGCGCCGGCTACCTCGCCGACGGCTATGCCCGGGCTTCCGGCCGGCCGGCTGCGGTCTTCGCGGCCGGA
CCCGGGGCGACCAACGTCGTGACCGCCGTGG
CCAATGCCTCGGTAAACCACGTACCCATACTCGTTCTCACCGGGGA
GGTGCCGGTTGCCGAATTCGGTCTGCATTCGCAGCAGGACACCAGCGAGGACGGGCTCGGCCTGGGTGCGGTCT
TCCGTCGCCTCTGCCGCAGCTCGGT
ATCGATCGAATCGGTGGCCAATGCTCGCACCAAGATCGACCGGGCGTTTC
GGGCCCTGGCGAGCACCCCCGGCGGACCGGTGCACATCAGCCTGCCGCGCGACCTGGTGGATGAGGTGCTGCC
CGACGACCCGGTCGCGATGACCGCCACCGGCCGCGCCGCCACCGCGATCCTCGCCCCGAGCGGTCCGCAGATC
GCCGCCGAGGTGATCGGCCGGCTGCAGCGATCCGCTGCGCCAATGCTGTTGCTGGGCAAC
GGTGCTCGCCGGGA
CGGCATCGGCGCCGGAATCCTCGCGCTGTGTGAGAAAGCCGGGCTGCCGTTTGCGACGACTCCCAACGGGCGCG
GAGTTGTCCGGAAACCGCATCGGCTTTTCTCGGCTGGTCTGGTGGTCTTCGGTGACGGCAGGGCCGAAGACTATC
TTTTCGGCCGCGCCGTGCGATCTGCTGATCGCGGTCGGCTCTCGTTCGACGGCTTGGTCACCCGCTCCTTTTCGC
CGCGCTGGAGCGCTCTGC
AAGCCGAGGTCATCCACGTCGACCCCGATCCCTCGGCGTTCGGCCGTTTGGTCCCG
ACCTCGCTAGGCGTCATTGCCACCGCGGGGCCTGGTGGAATCGCTGAGCGCCCGGACGGCGCGATCCGCGC
GAGCGGTTACGGTACCGGCGCGGGTTTCGTTGCCGGTCCCGCCAACCCGAGGCGACGCCATCCATCCGCTGGCG
GTCATGCGGGAGCTGGACTCCCTGTTGGCGTCGAACAGCACGCTCTGCACGGA
TTCCGGGACATGTATCTACTGG
GCCTTTCGCGGGATACCGGTGCGAGCGCCCGGAACGTTCTTTGCCACGGTCGATTTCGCCCCCATGGGCTGCGGT
GTCGTCGGCGCCATCGGCATGGCGCTGGCCCGGCCCGGCCAACGCGTCGTCTGCATCGCCGGTGACGGCGCGTT
CCTGATGCATGGCACCGAGGTCTCCACCGCGGTCGCCCAGGGCCTCCCGGTCACCTGGATTGTCTTGAACGACGG
ACAGTTAGTGAACCGACCAACCGGGGTTAGTACGGCCGAGGATGACGTTAATGATTCGGGTTCCCG
ATTGGTATGGCACTGGCACGTCCGGGTCAGCGTGTTGTTTGTATTGCCGGTGATGGTGCCTTTCTGATGCATGGCA
CCGAAGTTAGCACCGCAGTGGCACAGGGTCTGCCGGTTACCTGGATTGTGCTGAATGATGGTCAGCTGAGCGCAA
CCACCGCAGTTATTCGTGGTCGTATGGATCCGAGTCCTCTGGCAGCCACCGGTGCAAATGA
TCTGGCAGCAAAATGCACTGGGTGCACAGGGTATTCGTGTTGATAAAGCAGCAGATGTTCGTGCAGCCCTGGAAAAAGCACTGAT
TGCCACCGGTCCGTGTGTTGTTGATGTTGTTATTGATCCGGAACTGAACAAACCGGAAATTGGTGTTGGTAAATAAC
TCGAGCACCACCACACCAC

**myGE – amino acid sequence (codon optimized)**

MGSSHHHHHHSSGLVPRGSVMTVGDHLMVRMEAGISLCLGLPSRDLTLRALLARDDDFIDSVLRHETGGA
YLABDYGARASGPAVAFAGPGATNVVTATASANSNVPHVLPVTGVEPVAEFGLHSSQTDSEDLGLAPVAG
RCSVSSSIESANTVRKIRTLFSDRPVHSLPRVLDVPLDVPMATGTAALLAPSLGPSQIAVEVIGRL
QRSAAPMLLGGARRDGIAGILACEKALPFAPTNPNGRGRVPEHPSLVLGLFGDRAEYLFAPCDDLI
AVVGVSDFGLSFPSRWALSAQVEHPVHDPSFAPVPLVTSLGIAATRLGVESLATSARASARTVAPARSLP
VPPTRGDAIHPIVLREDLGLSLNCTDSCGCTICWYAFRRFIIVRAPGTFAVDFAMPNGCVVGAIGMALRP
GVRVCIAGDAGFMHGTEYSTVAVAGLPTVWILNDQLSATTAVIRGRMDPSLAATAGANDLAMANALGAQ
GIRVDRKADAAALREKALITAPCOPVDVVDNPRLKPEIGVK
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