Supplementary data

General Methods and Materials

Methods: NMR spectra were recorded on a Bruker Avance 400 spectrometer (^{1}H 400 MHz, ^{13}C 100 MHz) and are referenced internally according to residual solvent signal. The chemical shifts (δ) were recorded in ppm and the coupling constants (*J*) were reported in Hz, are corrected and refer to the apparent peak multiplicities. GC-FID spectra were recorded on Bruker Gas Chromatographer using Flame Ionization Detectors (FID). Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} plates (Merck). Flash column chromatography was performed on silica gel (60 Å, 40-60 micron) from Fluorochem.

Materials: All chemicals were of analytical grade purity and obtained from Sigma Aldrich (München, Germany), Merck (Darmstadt, Germany), VWR (Hannover, Germany) or Acros (Geel, Belgium). Restriction enzymes, 1kb plus protein marker and phusion polymerase were bought from Thermo Fisher Scientific (Waltham, MA, US), T4 ligase and NEBuilder® HiFi DNA assembly were purchased from New England Biolabs (Ipswich, MA, USA), oligos and sequencing services were purchased from Eurofins Genomics (Ebersberg, Germany). *Escherichia coli* DH5α and BL21(DE3) cells were purchased from New England Biolabs (Ipswich, MA, USA)

S.1 Expression of recombinant genes in *E. coli*

All ATA genes were codon-optimized and were kindly supplied by Prof. Nicholas Turner and his group from University of Manchester, UK, and Prof. Uwe Bornscheuer and his group from University of Greifswald, Germany. (*S*)-Selective ATAs (3HMU, 3I5T, Vibflu, ChrVio, *Pc*ATA, *Pp*ATA) and the (*R*)-selective ATAs (AspFum, NeoFis) were cloned into pET-22b. (*S*)-Selective *Pf*ATA was cloned in pET-28b and the (*R*)-selective MycVan and AspOry were cloned in pGASTON. Transformed *E. coli* BL21(DE3) cells were cultivated in 500 mL LB, supplemented with 100 µg mL^{-1} ampicillin or 50 µg mL^{-1} kanamycin, at 37 °C shaking at 200 rpm, starting with 5 mL inoculum of single-colony culture previously grown overnight. The culture was grown until the OD_{600} reached 0.5-0.7 and then expression was induced by addition of isopropyl β-D-1-thiogalactopyranoside (IPTG) (1 mM) or Rhamnose (5% w/v) and the cultures were incubated at 20 °C (VibFlu, ChrVio, AspFum, AspOry, NeoFis, MycVan) for 20 h or 30 °C (3HMU and 3I5T) for 6 h. Following incubation, cells were harvested by centrifugation (1450 x g, 20 mins) and stored at -20 °C.

Cell pellets were thawed, resuspended in lysis buffer containing potassium phosphate buffer (50 mM, pH 7), pyridoxal-5'-phosphate (0.1 mM) and 1X cocktail of proteases inhibitors and disrupted by sonication at 4 °C with 10 cycles of 30 s of sonication and 30 s of cooling at 40% amplitude, using QSonica model Q55. After centrifugation (24,000 rpm, 4 °C, 25 mins) in an Eppendorf 5424R microcentrifuge, the supernatant was clarified *via* filtration (0.45 µm filter) and used directly or purified on Äkta pure.

S.1.1 Protein determination and SDS-PAGE analysis

Total protein concentration in clarified and purified extracts was determined spectrophotometrically at 280 nm. The extinction co-efficient of each ATA at 280 nm (measured in water) was estimated by ExPASy ProtoParam tool (www.expasy.org).[1] SDS-PAGE analysis was carried out with 15% resolving and 5% stacking gel in Tris-glycine buffer system. The gel was stained with Coomassie Brilliant Blue G-250 and a broad range of protein marker (10-200 kDa) was used to determine the relative molecular weight.

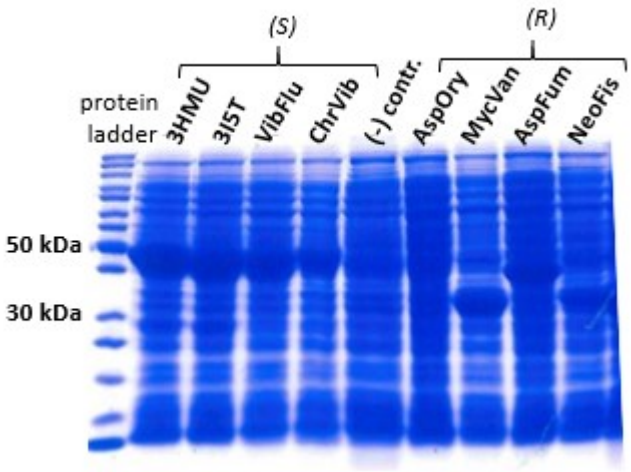


Figure S1. Sample SDS-PAGE of the clarified crude extracts of the ATAs.

S.1.2 Spectrophotometric assay

A kinetic assay developed from Schatzle *et al.*[2] was used as a standard enzymatic assay, employing pyruvate and *S*-phenylethylamine ((*S*)-PEA) as amine acceptor and donor, respectively. The reactions were carried out at 25 °C in HEPES buffer (1 mL, 50 mM, pH 8.0) containing pyruvate (2.5 mM), (*S*)-PEA (2.5 mM) and the appropriate amount of enzyme (0.04 to 4 µg purified enzyme or 0.5 to 900 µL of crude extract with an OD_{600} of 10, depending on the respective activity). The activity was determined by following the production of

acetophenone during the first two minutes of the reaction at 245 nm, using the spectrophotometer EPOCH2.

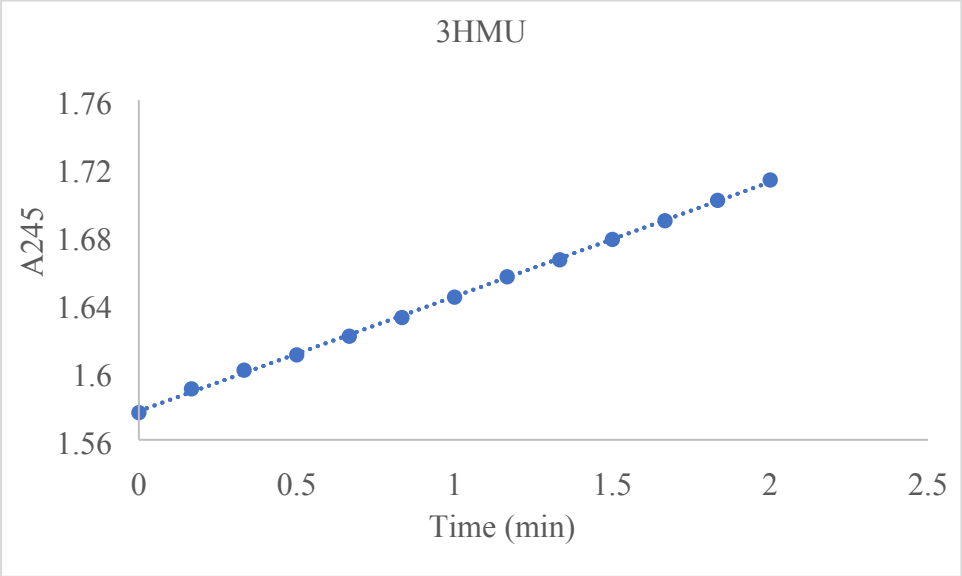


Figure S2. The increase in absorbance at 245 nm over time is measured, which corresponds to the amount acetophenone produced during the enzymatic assay.

S.1.3 Analytical scale biotransformations

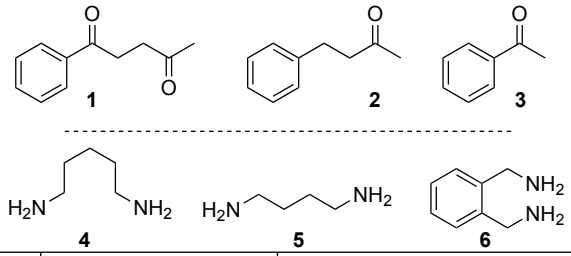
A solution of PLP (0.1 mM), ketone substrate (5 mM from a 500 mM stock in DMSO) and amine donor (1 or 3 equiv.) in phosphate (100 mM, pH 7-8), HEPES (100 mM, pH 7-8) or Tris buffer (100 mM, pH 9-10) was prepared and the pH was adjusted according to the enzyme employed. To this, clarified extract of the ATA preparation (1.5 mg mL^{-1}) was added and reactions were incubated at 30 °C in a gyratory incubator at 200 rpm. After 24 hours, the reactions were basified (pH 12), extracted with EtOAc (750 µL), derivatized with triethylamine 10 µL and acetic anhydride and analyzed by GC-FID.

GC Method

GC analysis was performed on a ThermoFisher 1310 chromatograph equipped with a flame ionizing detector, an AI 1310 autosampler and a CP-Chirasil-Dex-CB chiral column (25 m x 0.25 mm x 0.36 mm) and helium as a carrier gas. The front inlet temperature was set to 230 °C and the front detector was set to 250 °C. Split flow was set to 170 mL min^{-1} and the helium gas was set to a constant flow of 1.7 mL min^{-1}. The GC-FID temperature program for all compounds was the following; 40 °C hold for 2 min followed by 20 °C min^{-1} temperature rise

to 150 °C and then a hold for 5 minutes followed by a 30 °C min^{-1} temperature rise to 225 °C and a further hold for 8 min.

*Table S1. Conversion of ketones **1**, **2** or **3** (5 mM) to the corresponding chiral amine, utilizing the ATA as clarified crude extract (1.5 mg mL^{-1}), PLP (0.1 mM) and amine donor (5 mM), at 30 °C, 200 rpm, 24 h.*



Selectivity	Transaminase	pH	Acceptor	Conversion (%)			*ee* (%)
				Cadaverine **4**	Putrescine **5**	*o*-xylylene diamine **6**	
(*S*)	3HMU	9.0	**1**	75	72	74	99
			2	21	19	20	85
			3	nd	nd	nd	nd
	*Pc*ATA	9.0	**1**	43	20	23	99
			2	14	6	8	80
			3	nd	nd	nd	nd
	*Pf*ATA	9.0	**1**	54	28	35	99
			2	25	9	17	82
			3	9	nd	7	99
	*Pp*ATA	9.0	**1**	53	24	25	99
			2	18	9	11	85
			3	nd	nd	nd	nd
	3I5T	9.0	**1**	12	15	10	99
			2	14	10	7	80
			3	nd	nd	nd	nd
	ChrVio	7.0	**1**	2	nd	4	99
			2	8	2	7	99
			3	3	nd	3	99
	Vibflu	7.0	**1**	nd	nd	7	99
			2	nd	nd	6	99
			3	nd	nd	nd	nd
(*R*)	MycVan	7.5	**1**	nd	nd	4	99
			2	nd	nd	5	99
			3	nd	nd	nd	nd
	AspFum	7.5	**1**	nd	nd	nd	nd
			2	nd	nd	nd	nd
			3	nd	nd	nd	nd
	NeoFis	7.5	**1**	nd	nd	nd	nd
			2	nd	nd	nd	nd
			3	nd	nd	nd	nd

*Table S2. Conversions of 5 mM of **1** to the corresponding chiral amine utilizing purified extract of 3HMU, Pc, Pf or Pp ATA (1 mg mL^{-1}) in conditioned culture medium (GRLys1, NA6, Cada1) at 30 °C, 200 rpm, 24 h.*

C. glutamicum Culture	Transaminase	Conversion (%)		ee (%)
		1d	2d	
GRLys1	3HMU	15	15	99
	*pc*ATA	nd	nd	nd
	*pf*ATA	nd	nd	nd
	*pp*ATA	nd	nd	nd
NA6	3HMU	81	83	99
	*pc*ATA	75	75	99
	*pf*ATA	68	68	99
	*pp*ATA	71	71	99
Cada1	3HMU	55	98	99
	*pc*ATA	6	78	99
	*pf*ATA	6	70	99
	*pp*ATA	7	60	99

*Table S3. Conversion of **1** (5 mM) to the corresponding chiral amine, utilizing clarified crude extract (1.5 mg mL^{-1}) of 3HMU, Pc, Pf or PpATA, PLP (0.1 mM) and L-lysine (15 mM) at 30 °C, 200 rpm, 24h.*

Transaminase	**Conversion (%)**	***ee* (%)**
3HMU	16	99
*Pc*ATA	nd	nd
*Pf*ATA	nd	nd
*Pp*ATA	nd	nd

S.1.4 Protein purification

Purification of the ATAs was carried on Äkta Pure system (GE, Healthcare, Little Chalfont, UK). Clarified extract was loaded onto a 5 mL HisTrap column, washed with 10 column volumes of de-gassed phosphate buffer (50 mM, pH 8.0 containing PLP (0.1 mM) and imidazole (30 mM)) and eluted with de-gassed phosphate buffer (50 mM, pH 8.0 containing PLP (0.1 mM) and imidazole (300 mM)). Fractions of 5 mL were collected during the elution phase and SDS-PAGE gel was used to identify fractions containing the ATA. The protein solution was concentrated using VivaSpin 20, 50,000 MWCO PES (Sartorius, Gottingen, Germany) and the imidazole was removed *via* dialysis (tubing from Sigma Aldrich). Purified protein was analyzed by SDS-PAGE (found 95% pure) and instantly used for biotransformations.

S.1.5 Analytical scale biotransformations with conditioned medium

Cultures of *C. glutamicum* NA6 or Cada1 were grown (see section S.3) and an aliquot was taken after 24 h and 48 h. To the one-day or two-day mature culture media (1 mL) was added PLP (0.1 mM), 1-phenyl-1,4-pentanedione (5 mM from 500 mM stock in DMSO) and purified ATA extract (1 mg mL^{-1}), and the reaction mixture was incubated at 30 °C shaking at 200 rpm. After 24 hours the reactions were basified (pH 12), extracted with EtOAc (750 µL), derivatized with triethylamine (10 µL) and acetic anhydride (10 µL) and analyzed by GC-FID (see S.1.3).

S.1.6 pH study

The acetophenone assay described in S.1.2 was employed utilizing phosphate (100 mM, pH 7-8), HEPES (100 mM, pH 7-8) or Tris buffer (100 mM, pH 9-10), depending on the pH being analysed.

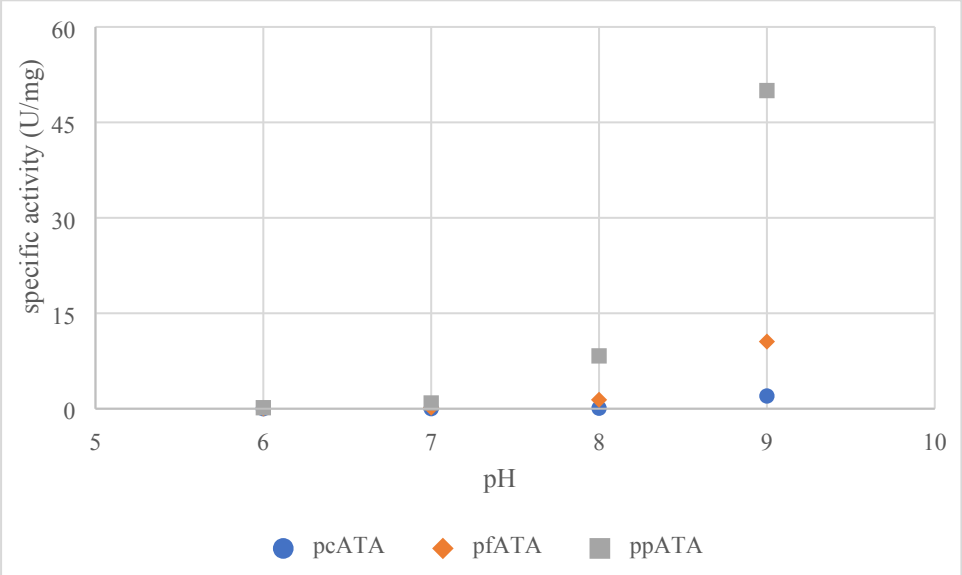


Figure S3. Measurement of the specific activity of purified extract of putrescine ATAs at different pH, utilizing the acetophenone assay.

S.2 Colony based solid-phase ATA assay

Colonies of transformed *E. coli* or *C. glutamicum* were grown at 30 °C overnight on LB agar, supplemented with appropriate antibiotic. Under sterile conditions, colonies were transferred onto a cellulose membrane, placed on top of filter papers soaked with IPTG (1 mM) for induction, and incubated at 30 °C for 24 h. Subsequently, the membrane was transferred onto filter papers soaked in a solution of *o*-xylylenediamine (10 mM) and pyruvate (10 mM) and incubated at room temperature. ATA activity was identified by the emergence of colored colonies, after 0.5, 8 and 24 h.

S.3 Expression of recombinant genes in *C. glutamicum*

Minimal media mCGXII[3] was used for the main cultures containing per liter distilled water: 20 g $(NH_4)_2SO_4$, 5 g Urea, 1 g KH_2PO_4, 1 g K_2HPO_4, 13.25 mg $CaCl_2{\cdot}2H_2O$, 0.25 g $MgSO_4{\cdot}7H_2O$, 10 mg $FeSO_4{\cdot}7H_2O$, 10 mg $MnSO_4{\cdot}H_2O$, 0.02 mg $NiCl_2{\cdot}6H_2O$, 0.313 mg $CuSO_4{\cdot}5H_2O$, 1 mg $ZnSO_4{\cdot}7H_2O$, 0.2 mg biotin, 42 g 3-(*N*-morpholino)-propane-sulphonic acid (MOPS) and 4% (w/v) glucose.

Multiple colonies of transformed *C. glutamicum* were used to inoculate 25 mL Brain Heart Infusion media enriched with sorbitol (5% w/v) (BHIS), supplemented with spectinomycin (100 µg mL^{-1}) and/or kanamycin (50 µg mL^{-1}) and grown at 30 °C overnight. Pellets were harvested *via* centrifugation (4000 rpm, 7 mins), washed with minimal medium mCGXII[3] (3 x 20 mL) and used to inoculate a 50 mL main culture, with starting OD_{600} of 0.5 (for NA6 or GRLys1) or 1.0 (for Cada1). The cultures were instantly induced with IPTG (1 mM), unless otherwise specified, and incubated at 30 °C shaking at 200 rpm. After 24 h, cells (or media) were either used directly or were harvested *via* centrifugation (4000 rpm, 7 mins).

S.3.1 Analytical-scale whole-cell biotransformations of diketone **1**. Evaluation of conversions achieved with various cell densities and results from reusability studies.

Transformants of *C. glutamicum* were grown as described above. After 24 h, the cells were either used directly or harvested *via* centrifugation (4000 rpm, 7 mins), when different cell densities were being evaluated. Harvested cells were resuspended in either media or HEPES buffer in different concentrations (50, 100, 150 and 200 mg mL^{-1}). To this, 1-phenyl-1,4-pentanedione (5 mM from 500 mM stock in DMSO) was added and the reaction mixture

was incubated at 30 °C shaking at 200 rpm. After 24 hours, the reaction was pelleted *via* centrifugation and the cells were reused, where specified (reusability studies). The supernatant was basified (pH 12), extracted with EtOAc (750 µL), derivatized with triethylamine (10 µL) and acetic anhydride (10 µL) and analyzed by GC-FID (see S.1.3).

*Table S4. Conversion of diketone **1** using GRLys1, NA6 and Cada1 strains transformed with Pc, Pf or PpATA in pEKEx3 at 30 °C or 37 °C, 200 rpm for 24h.*

Strain	Transaminase	Conversion (%)		*ee* (%)
		30 °C	37 °C	
GRLys1	*Pc*ATA	nd	nd	nd
NA6	*Pc*ATA	9	5	99
	*Pf*ATA	3	3	99
	*Pp*ATA	2	0	99
Cada1	*Pc*ATA	12	7	99
	*Pf*ATA	8	6	99
	*Pp*ATA	9	5	99

*Table S5. Conversion of ketone **1** using NA6, NA2b and GRLys1 strains transformed with Pc, Pf or PpATA in either pEKEx3 or pVWEx1, or PcATAx2 in pEKEx3, at 30 °C, 200 rpm for up to 48h.*

Strain[a]	Vector(s)[a]	Biocatalysts	Conversion (%)		*ee* (%)
			1d	**2d**	
NA6	pVWEx1 (for speC), pEKEx3 (for ATA)	speC, *Pc*ATA	9	12	99
		speC, *Pc*ATAx2	22	32	99
		speC, *Pf*ATA	3	4	99
		speC, *Pp*ATA	2	3	99
NA2b	pVWEx1 (for speC and ATA)	speC, *Pc*ATA	nd	7	99
		speC, *Pf*ATA	nd	5	99
		speC,*Pp*ATA	nd	5	99
GRLys1	pVWEx1 (for ldcC), pEKEx3 (for ATA)	ldcC, *Pc*ATA	12	17	99
		ldcC, *Pc*ATAx2	14	20	99
		ldcC, *Pf*ATA	8	8	99
		ldcC, *Pp*ATA	9	9	99
	pVWEx1 (for speC and ATA)	ldcC, *Pc*ATA	36	41	99
		ldcC, *Pf*ATA	32	35	99
		ldcC, *Pp*ATA	26	33	99

a: for exact genotypes see table S9*Table S6. Conversion of ketone **1** using different cell densities of GRLys1-pVWEx1-ldcC-PcATA in different media. N/A: not available*

Cell density	Conversion (%)		
	Media culture	HEPES buffer	HEPES buffer + 0.1 mM PLP
50 mg mL^{-1}	46	51	N/A
100 mg mL^{-1}	64	73	N/A
150 mg mL^{-1}	74	75	N/A
200 mg mL^{-1}	77	77	76

*Table S7. Conversion of **1** utilizing wet cell pellets (100 mg mL^{-1}) of C. glutamicum GRLys1-pVWEx1-ldcC-PcATA in three consecutive cycles.*

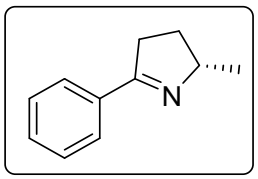
Cycle	**Conversion (%)**	***ee* (%)**
1	77	99
2	66	99
3	22	99

S.3.2 Preparative scale biotransformations of diketone 1

Multiple pre-cultures of *C. glutamicum* GRLys1 (50 mL) with the pVWEx1-*ldcC-Pc*ATA, in BHIS media were prepared. Two 400 mL cultures of mCGXII, supplemented with kanamycin

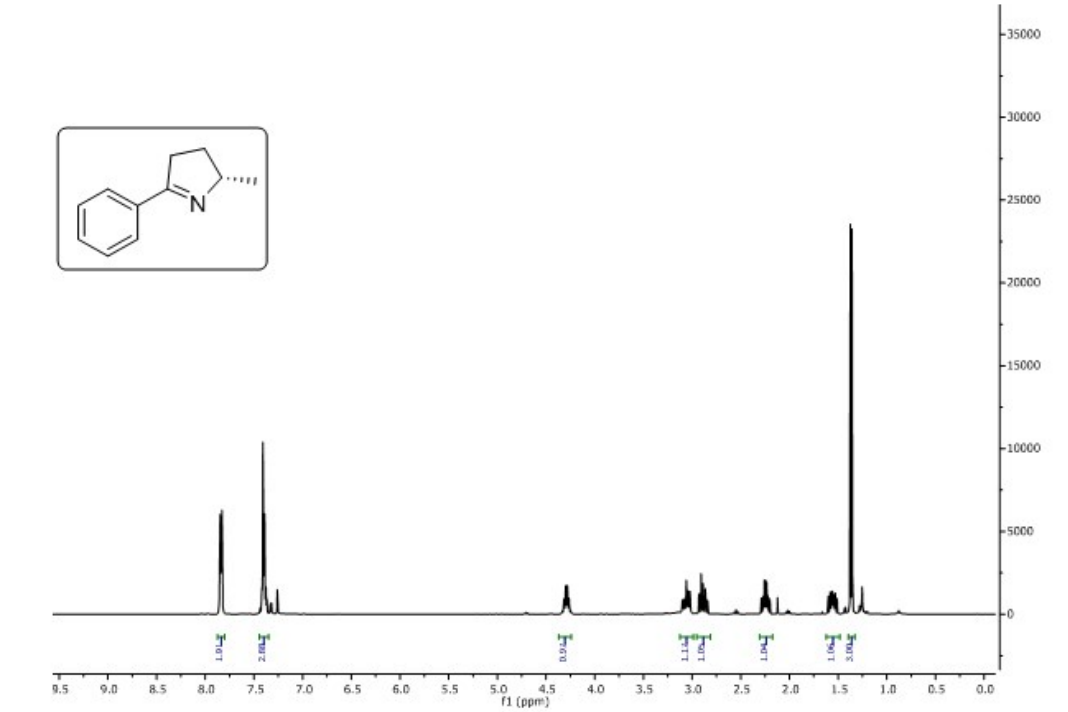
(50 µg mL^{-1}) and starting OD_{600} of 1.0 were prepared. Cultures were immediately induced with IPTG (1 mM) and incubated at 30 °C shaking at 200 rpm. After 24 h, the cells were pelleted *via* centrifugation (4000 rpm, 7 mins), washed with mCGXII (3 x 20 mL) and resuspended in HEPES buffer (140 mL, 100 mM, pH 8.0) at a final concentration of 150 mg mL^{-1} wet cell pellet. To this, 1-phenyl-1,4-pentanedione **1** (130 µL, 5 mM in 10 mL DMSO) was added and the reaction mixture was incubated at 30 °C shaking at 200 rpm. After 24 h, an aliquot was extracted and analyzed by GC-FID (see S.1.3). The reaction mixture was centrifuged (4000 rpm, 15 mins) and the supernatant was clarified *via* filtration (0.45 µm filter), basified with NaOH (pH 12.0) and extracted with EtOAc (4 x 50 mL). The combined organic extracts were dried over $MgSO_4$ and the volatiles were removed under reduced pressure. The mixture was purified in silica gel utilizing petrol:dichloromethane (10:90), affording **4** as a light yellow oil (50 mg, 42% yield, > 99% *ee*).

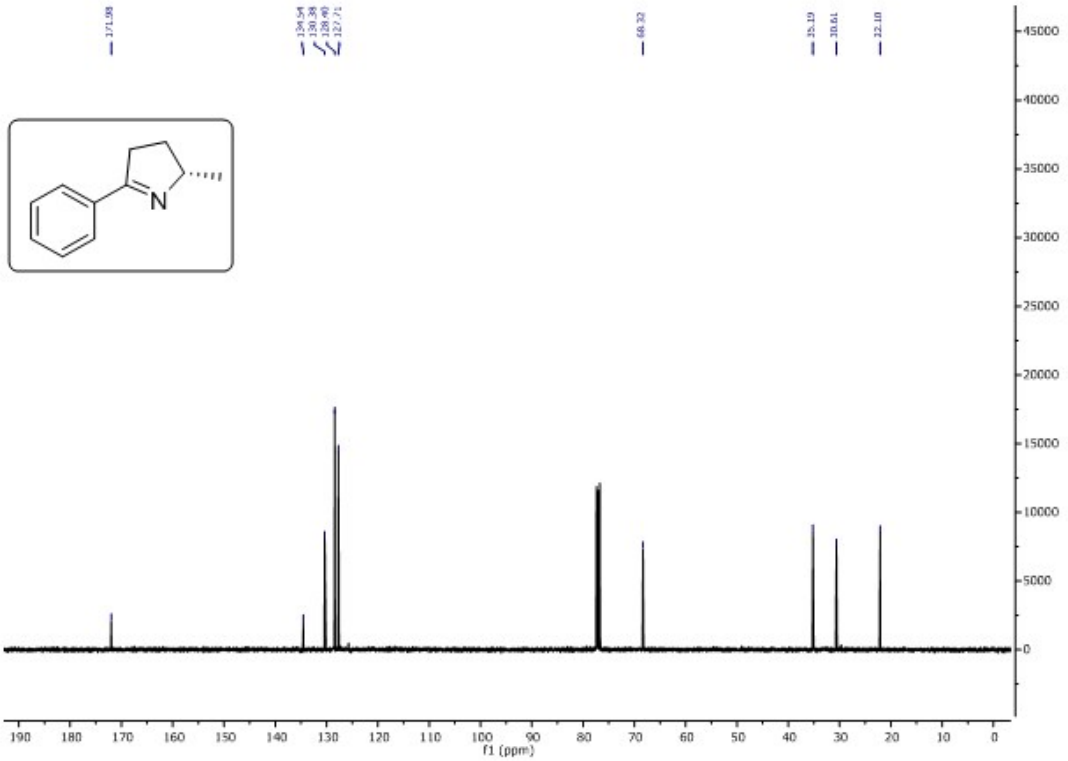
(*S*)-5-methyl-2-phenyl-1-pyrroline (4)[4]



Light yellow oil (50 mg, 42%, >99% *ee*). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.84 (dd, *J* = 7.5, 2.1, 2H), 7.44 – 7.35 (m, 3H), 4.35 – 4.25 (m, 1H), 3.06 (dddd, *J* = 16.9, 9.9, 4.8, 2.1, 1H), 2.89 (dddd, *J* = 16.9, 9.6, 7.7, 1.8, 1H), 2.25 (dddd, *J* = 12.5, 9.8, 7.7, 4.8 Hz, 1H), 1.59 – 1.52 (m, 1H), 1.37 (d, *J* = 6.8, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.0, 134.5, 130.4, 128.4, 127.7, 68.3, 35.2, 30.6, 22.1. MS (EI) m/z: calculated $C_{11}H_{14}N$ $[M+H]^+$ 160.1121; found: 160.1137. $[a]_D^{25} = -108.4$ (c 2.0, $CHCl_3$ lit: $^-$110.5). Data consistent with the literature.[4]

S.3.3 NMR-data





S.3.4 References

[1] E. Gasteiger, A. Gattiker, C. Hoogland, I. Ivanyi, R. D. Appel, A. Bairoch, *Nucleic Acids Res.* **2003**, *31*, 3784–3788.

[2] S. Schätzle, M. Höhne, E. Redestad, K. Robins, U. T. Bornscheuer, *Anal. Chem.* **2009**, *81*, 8244–8248.

[3] S. Unthan, A. Grünberger, J. van Ooyen, J. Gätgens, J. Heinrich, N. Paczia, W. Wiechert, D. Kohlheyer, S. Noack, *Biotechnol. Bioeng.* **2014**, *111*, 359–371.

[4] E. O'Reilly, C. Iglesias, D. Ghislieri, J. Hopwood, J. L. Galman, R. C. Lloyd, N. J. Turner, *Angew. Chemie - Int. Ed.* **2014**, *53*, 2447–2450.

S.4 Strains, plasmids and oligonucleotides used

Table S9. Strains and vectors used in this study

Strain or plasmid	Relevant characteristic	reference or source
E. coli DH5α	F⁻ *endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG purB20* ϕ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)U169, hsdR17(r_K^- m_K^+), λ⁻	New England Biolabs
E. coli BL21(DE3)	F⁻ *ompT gal dcm lon hsdS*$_B$($r_B^-m_B^-$) λ(DE3 [*lacI lacUV5-T7p07 ind1 sam7 nin5*]) [*malB*$^+$]$_{K\text{-}12}$(λ^S)	New England Biolabs
C. glutamicum strains		
ATCC 13032	Wild type strain naturally overproducing glutamate	American Type Culture Collection
GRLys1	*C. glutamicum* ATCC13032 with the following modifications: *Δpck*, *pyc*^{P458S}, *hom*^{V59A}, 2 copies of *lysC*^{T311I}, 2 copies of *asd*, 2 copies of *dapA*, 2 copies of *dapB*, 2 copies of *ddh*, 2 copies of *lysA*, 2 copies of *lysE*, in-frame deletion of prophages CGP1 (cg1507-cg1524), CGP2 (cg1746-cg1752) and CGP3 (cg1890-cg2071)	[1]
Cada1	GRLys1 carrying the pVWEx1-*ldcC* plasmid	this study
NA2	putrescine overproducing, L-arginine prototrophic strain derived from *C. glutamicum* ATCC13032 carrying in frame deletions of *argR* and *argF*, replacement of start codon GTG to TTG for chromosomal *odhA*, replacement of threonine codon 15 of chromosomal *odhI* by an alanine codon, carrying plasmid pVWEx1-*speC-argF*$_{21}$	[2]
NA2b	L-arginine auxotrophic, putrescine overproducing strain derived from NA2 carrying plasmid pVWEx1-*speC* instead of pVWEx1-*speC-argF*$_{21}$	This study
NA6	putrescine overproducing, L-arginine prototrophic strain derived from NA2 carrying in frame deletion of *snaA*, and plasmid pVWEx1-*speC-gapA-pyc-argB*$^{A49V/M54V}$-*argF*$_{21}$ instead of pVWEx1-*speC-argF*$_{21}$	[2]
Plasmids		
pET-22b	Amp-r; *E. coli* vector (PT7 *lacI*, pBR322-Ori)	lab stock
pET-22b-3HMU	derived from pET-22b, for regulated expression of 3HMU from *S. pomeroyi*	[3]
pET-22b-3I5T	derived from pET-22b, for regulated expression of 3I5T from *R. sphaeroides* KD131	[3]
pET-22b-ChrVio	derived from pET-22b, for regulated expression of ChrVio from *C. violaceum*	[4]
pET-22b-Vibflu	derived from pET-22b, for regulated expression of Vibflu from *V. fluvialis*	[5]

pET-22b-*Pc*ATA	derived from pET-22b, for regulated expression of *Pc*ATA from *P. chlororaphis subsp*. a*ureofaciens*	[6]
pET-22b-*Pp*ATA	derived from pET-22b, for regulated expression of *Pp*ATA from *P. putida*	[7]
pET-22b-AspFum	derived from pET-22b, for regulated expression of AspFum from *A. fumigatus*	[3]
pET-22b-NeoFis	derived from pET-22b, for regulated expression of NeoFis from *N. fischeri*	[3]
pET-28b	Km-r; *E. coli* vector (PT7 *lacI*, pBR322-Ori)	Lab stock
pET-28b-*Pf*ATA	derived from pET-28b, for regulated expression of *Pf*ATA from *P. fluorescens*	[6]
pRSET B	Amp-r; *E. coli* vector (PT7 *lacI*, pBR322-Ori)	[7]
pHEWT	derived from pHES-PUC, for regulated expression of HEWT from *H. elongata*	[7]
pGASTON	Amp-r; *E. coli* vector (Prha, pBR322-Ori)	[8]
pGASTON-MycVan	derived from pGASTON, for regulated expression of MycVan from *M. vanbaalenii*	[8]
pGASTON-AspORy	derived from pGASTON, for regulated expression of AspOry from *A. oryzae*	[9]
pEKEx3	Spec-r; *C. glutamicum*/*E. coli* (Ptac *lacI*^{q}, pBL1 ori)	[10]
pEKEx3-3HMU	derived from pEKEx3, for regulated expressions of 3HMU from *S. pomeroyi*	this study
pEKEx3-*Pc*ATA	derived from pEKEx3, for regulated expression of *Pc*ATA from *P. chlororaphis subsp. aureofaciens*	this study
pEKEx3-*Pc*ATAx2	derived from pEKEx3, for regulated expression of double insert of *Pc*ATA from *P. chlororaphis subsp. aureofaciens*	this study
pEKEx3-*Pf*ATA	derived from pEKEx3, for regulated expression of *Pf*ATA from *P. fluorescens*	this study
pEKEx3-*Pp*ATA	derived from pEKEx3, for regulated expression of *Pp*ATA from *P. putida*	this study
pVWEx1	Km-r; *C. glutamicum*/*E. coli* shuttle vector (Ptac *lacI*^{q}, pCG ori)	[11]
pVWEx1-*ldcC*	derived from pVWEx1, for regulated expression of *ldcC* from *E. coli* MG1655	this study
pVWEx1-*speC*	derived from pVWEx1, for regulated expression of *speC* from *E. coli* MG1655	this study
pVWEx1-*speC-gapA-pyc-argB*$^{A49V/M54V}$-*argF*$_{21}$	derived from pVWEx1 for the regulated expression of *argB*$^{A49V/M54V}$, *pyc* and *gapA* from *C. glutamicum*, *speC* from *E. coli* MG1655 and leaky expression of *argF*	[2]

pVWEx1-*ldcC*-*Pc*ATA	derived from pVWEx1, for regulated expression of *ldcC* from *E. coli* MG1655 and *Pc*ATA from *P. chlororaphis subsp. aureofaciens*	this study
pVWEx1-*ldcC*-*Pf*ATA	derived from pVWEx1, for regulated expression of *ldcC* from *E. coli* MG1655 and *Pf*ATA from *P. fluorescens*	this study
pVWEx1-*ldcC*-*Pp*ATA	derived from pVWEx1, for regulated expression of *ldcC* from *E. coli* MG1655 and *Pp*ATA from *P. putida*	this study
pVWEx1-*speC*-*Pc*ATA	derived from pVWEx1, for regulated expression of *speC* from *E. coli* MG1655 and *Pc*ATA from *P. chlororaphis subsp. aureofaciens*	this study
pVWEx1-*speC*-*Pf*ATA	derived from pVWEx1, for regulated expression of *speC* from *E. coli* MG1655 and *Pf*ATA from *P. fluorescens*	this study
pVWEx1-*speC*-*Pp*ATA	derived from pVWEx1, for regulated expression of *speC* from *E. coli* MG1655 and *Pp*ATA from *P. putida*	this study

Table S10. Primers used in this study

Primer Name	Sequence	Restriction site	Purpose
SG1_F	GCCTGCA*GTCGACCGAAAGGAGG ACAACC***ATG**AGCCTGGCGACCATTACG	SalI	Cloning of 3HMU
SG2_R	GGTTCTAGAGGAGCTC*GAATTC*GG ATCCTTATTAGTGG	EcoRI	Cloning of 3HMU
SG17_F	GGT*GTCGACCGAAAGGAGGACAACC* **ATG**ATCAGCAACAATCCGCAAACC	SalI	Cloning *Pc*ATA
SG18_R	GGTGGT*GAATTC*GCCGGATCTCAGTG GTG	EcoRI	Cloning *Pc*ATA
SG19_F	GGT*GTCGACCGAAAGGAGGACAACC* **ATG**GGCAGCAGCCATCATC	SalI	Cloning *Pf*ATA & *Pp*ATA
SG20_R	GGTGGT*GAATTC*TTAGCCTTGCAACG CACTGAGC	EcoRI	Cloning *Pf*ATA
SG21_R	GGTGGT*GAATTC*GCTACCGAATCGCC TCAAGG	EcoRI	Cloning *Pp*ATA
SG22_F	CAAGCTTGCATGCCTGCAG*GTCGAC*C *GAAAGGAGGACAAC*	SalI	HiFi assembly (pEKEx3, *Pc*ATA twice)
SG23_R	CGAACCACCGGTGGTTCATAGGTGGT GGTGGTG	-	HiFi assembly (pEKEx3, *Pc*ATA twice)
SG24_F	CCACCTATGAACCACCGGTGGTTCGA CC*GAAAGGAGGACAAC*	-	HiFi assembly (pEKEx3, *Pc*ATA twice)
SG25_R	CTGTAAAACGACGGCCAGTGTCATAG GTGGTGGTGGTG		HiFi assembly (pEKEx3, *Pc*ATA twice)

SG26_F	CCTGCAG*GTC*GAC<u>TCTAGAGGATTCC</u> *GAAAGGAGGCCCTTCAG***ATG**AACATC ATTGCCATTATGGG	SalI	HiFi assembly (pVWEx1-ldcC-*p*ATA) - amplify ldcC
SG27_R	CATGGTTGTCCTCCTTTCGTTATCCCG CCATTTTTAGGAC	-	HiFi assembly (pVWEx1-ldcC-*p*ATA) - amplify ldcC
SG28_F	CCTGCAG*GTC*GAC<u>TCTAGA</u>*GAAAGG AGGCCCTTCAG***ATG**AAATCAATGAA TATTGCCGC	SalI	HiFi assembly (pVWEx1-speC-*p*ATA) - amplify speC
SG30_F	TAAC*GAAAGGAGGACAACC***ATG**AT CAG	-	HiFi assembly (pVWEx1-ldcC/speC-*p*ATA) - amplify *Pc*ATA
SG31_R	ATTCGAGCTCGGTACCCGGGGATCTCA GCCCTGTAATGCACTCAACGTCAG	-	HiFi assembly (pVWEx1-ldcC-*Pc*ATA) - amplify *Pc*ATA
SG32_F	TAAC*GAAAGGAGGACAACC***ATG**ACCC GCAATAACCCGCAAAC	-	HiFi assembly (pVWEx1-ldcC/speC-*p*ATA) - amplify *Pf*ATA
SG33_R	ATTCGAGCTCGGTACCCGGGGATCTT AGCCTTGCAACGCACTGAGC	-	HiFi assembly (pVWEx1-ldcC-*Pf*ATA) - amplify *Pf*ATA
SG34_F	TAAC*GAAAGGAGGACAACC***ATG**AGCA CCAACAACCCGCAAAC	-	HiFi assembly (pVWEx1-ldcC/speC-*p*ATA) - amplify *Pp*ATA
SG35_R	ATTCGAGCTCGGTACCCGGGGATCCT ACCGAATCGCCTCAAGGGTC	-	HiFi assembly (pVWEx1-ldcC-*p*ATA) - amplify *Pp*ATA

Restriction sites are underlined, start codons are bold and ribosomal binding sides including spacer are italicized

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