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Green Chemistry

COMMUNICATION

Biocatalytic transamination with near-stoichiometric inexpensive amine donors mediated by bifunctional mono- and di-amine transaminases§

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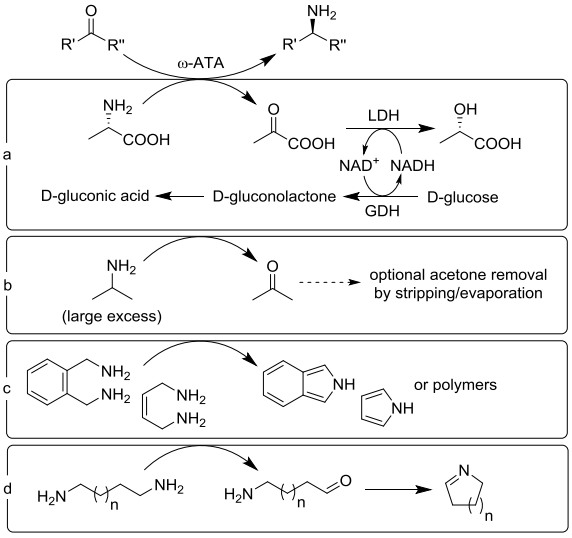
The discovery and characterisation of enzymes with both monoamine and diamine transaminase activity is reported, allowing conversion of a wide range of target ketone substrates with just a small excess of amine donor. The diamine co-substrates (putrescine, cadaverine or spermidine) are bio-derived and the enzyme system results in very little waste, making it a greener strategy for the production of valuable amine fine chemicals and pharmaceuticals.

Biocatalytic strategies for the synthesis of high added-value optically pure chiral intermediates provide extraordinary benefits to the pharmaceutical and agrochemical industries, due to the excellent activity, stereo-/regioselectivity and stability of many biocatalysts, often unmatched by their corresponding chemical equivalents.[1] There are obvious sustainability benefits to such approaches, including renewable resourcing of recombinant biocatalysts, solvent- and metal-free chemical transformations and ambient temperature/pressure of operation. In spite of this highly cited "greenness", the concept of any given biocatalytic transformation being effectively environmentally benign has been questioned in several instances.[2] Many widely used biotransformation protocols require, for example, expensive cofactor supplementation and/or large excesses of co-substrate to combat poor atom efficiency and unfavourable equilibria.

The asymmetric amination of ketones to enantiomerically enriched amines mediated by ω-amine transaminases (ω-ATAs, E.C. 2.6.1.18) is undoubtedly one of the most studied and exploited biotransformations which has become integrated into modern industrial practice. The broad substrate tolerance of this class of enzymes is one of their most appealing features in synthetic applications. A prominent example of this is the commercial manufacture of sitagliptin – probably one of the most cited success stories of industrial biocatalysis.[3]

For these reactions, the thermodynamic equilibria are often unfavourable and hence different methods for shifting the equilibrium have been sought to address this long-standing challenge (Scheme 1).[4] One of the earliest approaches, still heavily relied on, was the coupling of the enzymatic reaction with a secondary concomitant irreversible reaction (e.g., conversion of pyruvate by-product to L-lactate with lactate dehydrogenase, paired with an oxidoreductase to recycle NAD(P)H, Scheme 1a).[5,6] Whilst producing naturally-degradable biochemicals as by-products, this method has the obvious disadvantage of requiring two additional enzymes and an expensive cofactor to achieve high conversion of the starting ketone.



Scheme 1. Different approaches to shifting the thermodynamic equilibrium of ω-ATA transaminations.

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§ This manuscript is dedicated to Professor Romas Kazlauskas on the occasion of his 60th birthday.

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On the other hand, for industrial scale application, the approach of choice is the use of a sacrificial amine donor in large excess to drive the equilibrium towards the desired product. Isopropylamine is widely used for this purpose, because of its effectiveness and availability.[7] This approach has proven very cost-efficient in many cases, however, a 100-fold excess (or higher) is required to drive equilibrium. Also, in order to improve the yields, the removal of the volatile acetone waste product by evaporation or stripping needs to be implemented using specialised temperature platforms and heat-stable enzymes.[8]

More recently, the use of synthetic diamines such as *o*-xylylenediamine[9] and but-2-ene-1,4-diamine[10] has been the focal point to displace the equilibria of ω-ATA reactions by spontaneous cyclisation and subsequent ring aromatisation of the aminocarbonyl by-product (Scheme 1c). However, these donors are usually expensive, highly toxic and often form difficult to remove polymers following aromatisation, adding to downstream processing costs.

In contrast, biogenic terminal diamines present themselves as renewably-sourced alternative amino donors. In addition to their simple bio-based production and relatively low cost, upon transamination they are converted into reactive amino aldehydes which spontaneously convert to cyclic imines, thus driving the equilibrium towards amination of ketones. Enzymes active on such compounds, known as α,ω-diamine transaminases (α,ω-DTAs), have exploited these advantages for the synthesis of *N*-heterocycles precursors.[11] However, these enzymes were found to have strict preference for pyruvate over other ketones, making them unsuitable for the synthesis of a broad range of amines. Nevertheless, if the substrate specificity could be addressed, biogenic diamines would offer a distinct approach to equilibrium issues (Scheme 1d), intrinsically "greener" than previous ones, since near-stoichiometric loadings of sacrificial diamines could be applied, in principle.

Herein, we report the discovery and characterisation of a panel of bifunctional α,ω-DTA/ω-ATA enzymes that readily accept cheap and easily-accessible mono-/diamine donors as well as possessing broad ketone acceptor scope, allowing an equilibrium shift to reach theoretical yield of 100% aminated product with almost stoichiometric donor loadings.

Results and discussion

The lack of activity of commonly used ω-ATAs towards simple aliphatic diamines such as putrescine (1,4-diaminobutane, **1a**), cadaverine (1,5-diaminopentane, **1b**) and spermidine (1,8-diamino-4-azaoctane, **1c**) has been previously reported.[10,11] Indeed, the only relevant example is a single commercial transaminase (ATA256) recently found through extensive screening of a large biocatalyst panel from Codexis, highlighting the rarity of such activity.[12] This transaminase was shown to have activity with a narrow range of ketone acceptors, and its commercial nature precludes evolutionary, sequence and structural insight into its substrate scope or optimisation through engineering. To address this gap in the transaminase toolbox, we undertook a search for new candidate enzymes able to accept diamine donors.

As a promising starting point for the development of an enzyme with broader substrate range we identified the putrescine transaminase gene *spuC*, part of the polyamine uptake and utilization pathway in *Pseudomonas aeruginosa* PAO1.[13] The *spu* (spermidine utilisation) operon, consisting of 9 genes, was previously characterised as responsible for spermidine transport and the catabolic route of putrescine in the arginine decarboxylase pathway. Genes with analogous functions are found in *E. coli* such as the complementary *ygjG* gene encoding for putrescine transaminase that suggests a common pathway between the two microorganisms.[14]

To probe the evolutionary relationships between known putrescine transaminases and well-characterised ω-TAs, a multiple sequence alignment was performed, from which a cladogram could be constructed to infer the order of divergence events (Figure 1). Interestingly, it was found that sequences with reported diamine transaminase activity clustered within the ω-ATA sequences as polyphyletic groups. SpuC from *P. aeruginosa* (PA-SpuC) was revealed to have high sequence identity with the well-studied Cv-ATA from *Chromobacterium violaceum* (55%)[15,16] and only 24% identity with the more functionally similar *E. coli* YgjG protein.[14] This indicated two separate acquisitions of such activity in this family, with one convergence (Pa-SpuC) occurring relatively recently in evolutionary history compared to the other. As previous reports with the more ancient putrescine transaminases (Ec-, Bme- and Bmy-YgjG) revealed minimal acceptance of monoamine substrates,[11] it is probable that any ancestral ω-TA function of these enzyme has been lost. Due to the higher sequence similarity between the Pa-SpuC and characterised ω-ATA biocatalysts, this enzyme was chosen for investigation of potential latent ω-ATA side-activity. Previous experiments carried out with Pa-SpuC revealed that, although it was likely to be involved in the catabolism of diamines,[13] minimal activity was observed with putrescine and derived amides.[17] In light of this, a selection of predicted orthologues were mined from publically-available sequence data, cloned and expressed to access the natural diversity of SpuC enzymes with respect to activity and potential monoamine/diamine acceptance.

A Basic Local Alignment Search Tool (BLAST) analysis of the characterised *spuC* gene from the pathogenic *P. aeruginosa* PAO1 strain revealed other homologous genes from

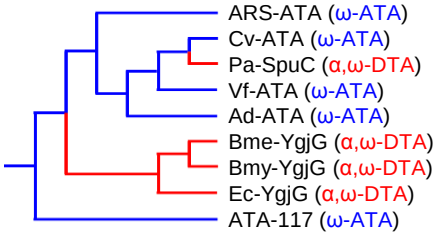
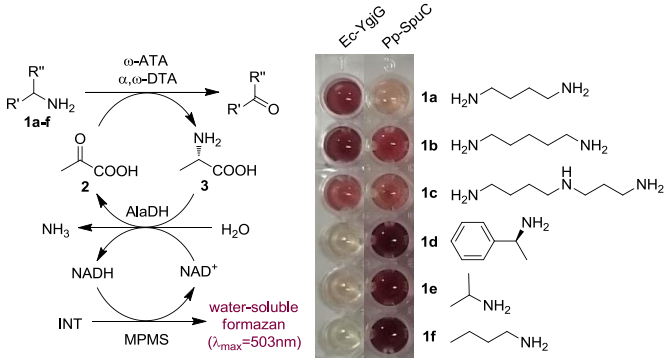


Figure 1. Cladogram showing evolutionary relationships between various wild-type transaminase enzymes as inferred from amino acid sequence identity.

Pseudomonas species, in particular *P. putida* (Pp-*spuC*), *P. chlororaphis* subsp. *aureofaciens* (Pc-*spuC*) and *P. fluorescens* (Pf-*spuC*) all with an aligned protein sequence identity of 69% (UNIPROT). The three putative *spuC* genes were cloned from our in-house NCIMB culture collection and subcloned into apET-28b vector followed by overexpression in *E. coli* BL21(DE3) cells. All three enzymes were successfully overproduced and purified as recombinant N-terminal His_6-tagged protein for screening against a representative panel of diamine and monoamine donors.

In order to determine the activity of the SpuC enzymes, an adapted version of the previously described L-amino acid dehydrogenase colorimetric assay[18] was employed, using pyruvate as the amino acceptor, L-alanine dehydrogenase from *Bacillus megaterium* to regenerate pyruvate with concomitant reduction of NAD^+, and the phenazine-tetrazolium system (MPMS+INT) for colour development (Scheme 2). The results for amines **1a-f** are summarised in Table 1 (additional mono- and diamines were tested, and kinetic constants were determined, see ESI).



Scheme 2. Activity assay used for the characterisation of ω-ATAs and α,ω-DTAs.

Table 1. Specific activities of the novel SpuC enzymes against amine donors **1a-f**, compared with Ec-YgjG and Cv-ATA.

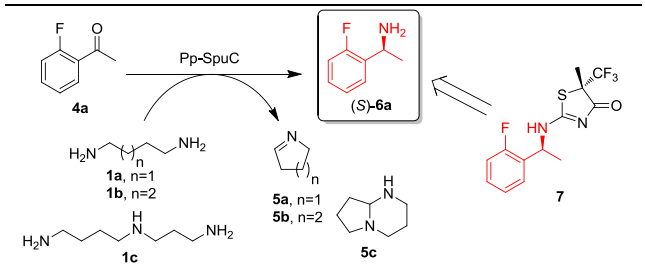
	Specific activity (U/mg)[a]				
Subs.	Pp-SpuC	Pf-SpuC	Pc-SpuC	Ec-YgjG	Cv-ATA
1a	1.3	0.4	1.4	2.7	n.d.
1b	27.3	16.5	15.1	2.7	7.7
1c	15.7	16.5	21.5	2.1	n.d.
1d	23.2	25.7	30.4	n.d.	28.9
1e	20.4	18.5	18.7	0.05	5.6
1f	16.5	19.3	13.5	n.d.	14.1

Expt. Cond.: 5 mM **1a-1f**, 1 mM **2**, 1 mM PLP, 0.2 mg mL^{-1} purified ATA, 100 mM sodium phosphate buffer, pH 8.0, 30°C. a: one unit of enzyme activity was defined as the amount of enzyme that produced 1 µmol of alanine per min at 30°C. n.d.: not determined (too low activity).

Our initial findings showed that all the recombinant SpuC proteins displayed high activity with the biogenic diamine donors **1a-1c** of interest with comparable specific activities within the different *Pseudomonas* species. Despite a 2-fold increase in activity of Ec-YgjG with putrescine **1a**, Pp-SpuC exhibited a 10-fold greater activity with cadaverine **1b**, Pc-SpuC with spermidine **1c**, with little or no activity displayed with Cv-ATA. Surprisingly, the SpuC enzymes also gave promiscuous activity with industrially relevant monoamines (*S*)-methylbenzylamine **1d**, isopropylamine **1e** and butylamine **1f**, which have been extensively studied with ω-ATAs, but none has been reported to have high activity with biogenic amines. Further studies on the diaminoalkane series was in close agreement with Cv-ATA as the carbon backbone increased in size (>C_6), in contrast to the diminished activity presented in Ec-YgjG (see ESI). This pattern is in part due to the narrow hydrophobic channel approaching the enzyme active site elucidated from recent crystal structures.[19]

Encouraged by these preliminary studies, we turned to the investigation of non-keto acid prochiral acceptors for preparative applications. As a model keto acceptor for reaction optimisation we chose *o*-fluoroacetophenone **4a**, since the chiral amine (*S*)-**6a** produced after transamination can be coupled to rhodanine scaffolds resulting in heterocycle analogues (e.g., **7**) with a range of pharmacological activities such as the clinical treatment of type II diabetes mellitus.[20,21] Biotransformations were conducted using our SpuC enzymes using different amounts of the diamine donors **1a-c**. The best conversions (Table 2) were obtained with Pp-SpuC (that also showed the highest specific activity for **1a-b** before), although the values for Pf-SpuC and Pc-SpuC were very similar.

Table 2. Testing of different diamine loadings in the transamination of **4a** mediated by Pp-SpuC.



Diamine	**1a**		**1b**		**1c**	
Equiv.	C (%)[a]	ee (%)[a]	C (%)[a]	ee (%)[a]	C (%)[a]	ee (%)[a]
1.5	84	>99	95	>99	83	>99
5	91	>99	96	>99	78	>99
10	87	>99	95	>99	73	>99
25	77	>99	83	>99	63	86

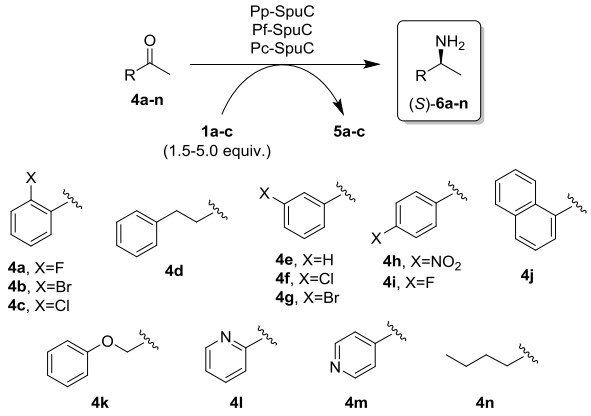
Expt. Cond.: 5 mM **4a**, 1 mM PLP, 1% v/v DMSO, 2 mg mL^{-1} purified SpuC, 50 mM HEPES buffer, pH 9.0, 40°C, 250 rpm, 24 h. a: measured by HPLC on a chiral stationary phase. C = conversion. ee = enantiomeric excess.

Remarkably, high conversions were observed at near-stoichiometric amounts of diamine donors **1a-1c**, indicating an efficient displacement of the equilibrium via *in situ* cyclisation, providing a greener substitute than 50 equivalents of volatile isopropylamine. The transamination of **1a-b** gave ω-aminoaldehydes which underwent spontaneous cyclisation to 1-pyrroline **5a** and 1-piperideine **5b**, respectively (as confirmed by GC-MS). Biotransformations were conducted at pH 9.0 and it was previously shown via ^{1}H NMR that the imine monomers are stable at conditions between pH 7-13.[22] Amino donor **1c**, instead, afforded the corresponding amino-imine that spontaneously cyclises a second time to yield the fused bicyclic structure **5c** under biotransformation conditions. Interestingly, increasing the concentration of **1c** not only did not improve

the conversion to **6a**, it was even detrimental, possibly due to inhibition by the *N*-heterocycle side-product. The optical purity of the product was almost invariably >99% with all three enzymes tested.

In order to test the feasibility of larger scale processes we also performed a preparative biotransformation with **4a** and 2 equivalents of **1b**, purifying the product by column chromatography after extraction. The procedure afforded (*S*)-**6a** with an isolated yield of 79% and >99% ee.

Finally, to prove the wide applicability of this procedure, we also tested a panel of prochiral ketones **4a-n** that afford synthetically useful chiral amines of interest for the production of fine chemicals and pharmaceuticals (Scheme 3). Even though the monomeric imines **5a** and **5b** are known to oligomerise in aqueous environment,[12] the corresponding products (as well as the bicyclic product **5c**) are water soluble and do not interfere with the standard work-up. Therefore, the chiral amine products of the transamination **6a-n** can be easily recovered by extraction (together with residual ketone) for HPLC analysis. The highest conversions obtained for each substrate among the three SpuC enzymes are listed in Table 3. The (*S*)-enantiomer of the product was formed with all substrate/enzymes pairs, most often with perfect enantioselectivity. Only substrates **4d**, **i**, **k** and **n** afforded in some cases slightly lower ee values (56-96%), however, at least one of the three SpuC enzymes tested provided >99% ee. This emphasises the importance of screening several different members of the family for each application.



Scheme 3. Panel of aromatic and aliphatic ketones tested with SpuC enzymes.

The sterically hindered *ortho*-substituted ketone substrates **4a-c** afforded high conversions with high enantioselectivities (>99%). This high conversion is presumably due to a stabilising interaction between the amine formed and the halogen atom at the *ortho*-position, that drives the transamination reaction more readily,[23] than solely based on the electrostatic activation of the halogen substituent (as evident from the contrasting low conversion attained for the *para*-fluoro derivative **4i** and the high conversion for the *para*-nitro derivative **4h**). Likewise, a similar stabilising effect of the intramolecular *H*-bond with the newly formed amine and vicinal oxygen atom of **4k** has been suggested,[24] that would account for the good conversion (41-59%) of this substrate.

Table 3. Amination of ketones **4a-n** with diamine donors **1a-c** (only the best conversion obtained among the three SpuC enzymes is shown).

	Diamine **1a**			Diamine **1b**			Diamine **1c**		
Subs.	SpuC	C (%)[a]	ee (%)[a]	SpuC	C (%)[a]	ee (%)[a]	SpuC	C (%)[a]	ee (%)[a]
4a	Pf	92	>99	Pc	97	>99	Pc	97	>99
4b	Pc	89[b]	>99	Pc	93	>99	Pc	93	>99
4c	Pf	75	>99	Pc	91	>99	Pc	96	>99
4d	Pf	34[b]	75	Pc	72[b]	74	Pp	47	>99
4e	Pf	12[b]	>99	Pp	42[b]	>99	Pc	30	>99
4f	Pc	35[b]	>99	Pc	62[b]	>99	Pc	74	>99
4g	Pp	63[b]	>99	Pp	80[b]	>99	Pc	86	>99
4h	Pc	99[b]	>99	Pc	93	>99	Pc	99	>99
4i	Pp	12	>99	Pc	35	>99	Pf	14	>99
4j	Pc	37[b]	>99	Pc	80[b]	>99	Pc	38	>99
4k	Pp	92	>99	Pc	87	>99	Pf	81	>99
4l	Pp	79	>99	Pp	96	>99	Pf	91	>99
4m	Pp	62[b]	>99	Pp	95	>99	Pp	55	>99
4n	Pp	99[b]	92	Pf	99[b]	>99	Pp	91	95

Expt. Cond.: 5 mM **4a-n**, 1.5 equiv. **1a-c**, 1mM PLP, 1% v/v DMSO, 2 mg mL^{-1} purified SpuC, 50 mM HEPES buffer, pH 9.0, 40°C, 250 rpm, 24 h. a: determined by HPLC on a chiral stationary phase. b: in these cases 5. equiv. of amine donor were used to increase the conversion values. C = conversion. ee = enantiomeric excess.

For non-activated ketone substrates (**4d-g**, **j** and **n**) the conversions appeared considerably lower, therefore we tested also a slightly higher loading of amine donors **1a-b** (5 equiv.), leading to modest to excellent conversions. Interestingly, however, with spermidine **1c** comparable conversions were reliably obtained at near-stoichiometric amounts (1.5 equiv.) compared to the better amino donor **1b** (5 equiv.). This effect can be rationalised by the higher stability of the bicyclic amine product **5c**.

A few examples of the pharmaceutical relevance of the chiral amine products thus obtained are several *N*-Methyl-D-aspartate (NMDA) glycine-site antagonists used in the treatment neuropathic pain (*i.e.*, Parkinson's disease), synthesised from (*S*)-**6l-m**,[25] or the analogues of the antiarrythmic mexiletine obtained from (*S*)-**6k**.[26] All these compounds were obtained in almost quantitative conversion and excellent enantioselectivity (Table 3).

Conclusions

In summary, we report the identification, heterologous production and characterisation of SpuC orthologues, a class of transaminases that uniquely allow the conversion of numerous ketone substrates at the expense of natural diamines. The enzymes were found to afford high conversion of a broad range of substrates with a modest excess of amine donor, giving good to excellent enantiomeric excess values for pharmaceutically relevant chiral amines.

The application of these bifunctional SpuC enzymes effectively combines the appealing substrate breadth of traditional ω-ATAs with the equilibrium shifting potential of diamine donor acceptance by α,ω-DTAs. This shift, requiring just 1.5-5.0 equivalents of bio-derived putrescine or cadaverine, represents a clean, 'green' and effective synthetic strategy, as opposed to the widespread use of a 50-fold excess of conventional, often poorly-accepted monoamines. The

method also mitigates the need for expensive cofactor supplementation and complex regeneration systems often employed in conjunction.

The approaches developed in this work are particularly relevant to recent advances in metabolic engineering of host strains for enhanced fermentation of putrescine,[27] potentially enabling the creation of an integrated microbial cell catalyst for chiral amine synthesis.

Acknowledgements

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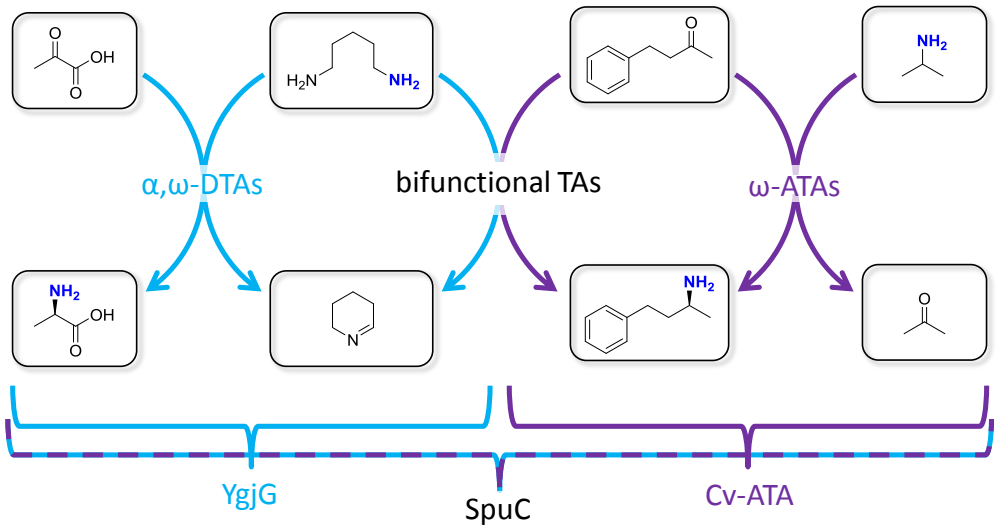

Table of contents text:
Bifunctional transaminases enable a strategy for the production of chiral amines using small excesses of diamine donors.

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ELECTRONIC SUPPLEMENTARY INFORMATION (ESI)

Biocatalytic transamination with near-stoichiometric inexpensive amine donors mediated by bifunctional mono- and di-amine transaminases

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Table of contents

View Article Online
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I. General methods and materials

1.1 Materials

Commercially available reagents were used throughout without further purification. Putrescine and all other reagents were purchased from Sigma Aldrich (St Louis, MO, USA) or Acros including anhydrous solvents. Restriction enzymes, T4 ligase, *taq* polymerase, dNTPs and broad protein marker (2-212 kDa) were purchased from New England Biolabs (Ipswich, MA, USA). *Escherichia coli* DH5α and BL21 (DE3) cells were purchased from New England Biolabs (Ipswich, MA, USA). Expression vector pET-28b was purchased from Novagen (Darmstadt, Germany) and was used for gene expression. *Chromobacterium violaceum* (Cv-TA) was purchased from LG standards (ATCC 12472, NCIMB 9178). *Pseudomonas florescens (Asc no. 13500), Pseudomonas chlororaphis subsp. aureofaciens 30-84*, (Asc. no 9392), *Pseudomonas putida* NBRC 14161 (Asc no. 9494), and *Bacillus megaterium* (Asc. No 9376) were purchased from the NCIMB culture collection (Aberdeen, UK).

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II. Molecular biology protocols

2.1 Cloning of spuC genes from Pseudomonas strains:

The coding region of the *spuC* gene was amplified by colony PCR from *Pseudomonas putida* NBRC 14161, P*seudomonas chlororaphis subsp. aureofaciens 30-84*, and *Pseudomonas fluorescens* using the primers detailed in Table S1.

Table S1. Primers for cloning the spuC gene from *Pseudomonas putida* NBRC 14161, *Pseudomonas chlororaphis subsp. aureofaciens 30-84*, and *Pseudomonas fluorescens.*

Pf-spuC	(5'-CAGCCATATGATGACCCGCAATAACCCGCAAACCCGTGAA-3') (5'-CACCTCGAGTTAGCCTTGCAACGCACTGAGCGTCAGGTCC-3')
Pp-spuC	(5'- AGTCGACATATGAGCACCAACAACCCGCAAACCCG-3') (5'- ATTCGGCTCGAGCTACCGAATCGCCTCAAGGGTC-3')
Pc-spuC	FW: (5'- GCAAGCATATGAYCAGCAACAAYCCGCAAACCCGTG-3') RV: (5'- GCAAGCTCGAGTTAGCCCTGYAAYGCACTCARSGTC-3')

The *NdeI* and *XhoI* restriction sites are underlined. The following PCR protocol was used: 5 min denaturation at 95 °C and then 30 cycles of 30 s denaturation at 95 °C, 30 s annealing at 58 °C and 90 s elongation at 68 °C with a 5 min final extension time at 68 °C. The PCR product was cloned into ZERO Blunt TOPO PCR cloning vector (K2830-20) following the manufacture's protocol.

The alanine dehydrogenase (Aldh) gene from *Bacillus megaterium* was amplified using forward (5'-GCAGCCATATGATTATTGGCGTACCAAAAGAAATC-3') and reverse (5'-GCAAGCTCGAGTTAGAT-AGAAGCTAATTCTTTTTCAAGAG-3') primers (XhoI/*NdeI* restriction sites underlined) and was amplified and cloned into TOPO PCR cloning vector as above.

Pf-spuC and *Aldh* genes were subcloned into pET-28b expression vector containing an *N*-terminal His_6 tag with a thrombin linker. Pc-spuC and Pp-spuC, was subcloned into pET-22b expression vector containing a *C*-terminal His tag with a thrombin linker. The inserted genes were in-frame downstream from the ribosome binding site as confirmed via DNA sequencing (Eurofins).

Table S2. Sequence-identity matrix heat map of the transaminases used to draw the cladogram in Figure 1.

[%]	ATA-117	ARS-ATA	Ec-YgjG	Bme-YgjG	Bmy-YgjG	AdATA	VfATA	CvATA	PaSpuC
ATA-117	100.0	16.7	17.0	18.1	17.7	16.4	15.0	16.1	15.5
ARS-ATA	16.7	100.0	25.6	27.8	29.2	25.8	22.9	25.7	28.3
Ec-YgjG	17.0	25.6	100.0	61.5	60.0	28.8	25.0	26.7	28.3
Bme-YgjG	18.1	27.8	61.5	100.0	85.8	29.8	26.0	27.9	28.4
Bmy-YgjG	17.7	29.2	60.0	85.8	100.0	30.3	24.6	25.9	28.7
AdATA	16.4	25.8	28.8	29.8	30.3	100.0	28.9	32.8	35.9
VfATA	15.0	22.9	25.0	26.0	24.6	28.9	100.0	37.3	38.3
CvATA	16.1	25.7	26.7	27.9	25.9	32.8	37.3	100.0	59.6
PaSpuC	15.5	28.3	28.3	28.4	28.7	35.9	38.3	59.6	100.0

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III. Expression and purification of HisTag recombinant proteins

3.1 Protein expression protocols

The *spuC* genes from *Pseudomonas putida* NBRC 1416 and *Pseudomonas chlororaphis subsp. aureofaciens 30-84*, were transformed into *E. coli* BL21 (DE3) for yielding *E. coli* BL21 (pET-22b(+)4–Pp_spuC) and *E. coli* BL21 (pET-22b(+)4–Pc_spuC). Similarly, the *spuC* gene from *Pseudomonas fluorescens* F113 in pET-28b(+) expression vector and the *ald* gene from *Bacillus megaterium* DSM 319 in pET-22b(+)-4 expression vector were used to yield *E. coli* BL21 (pET-28b(+)–Pf_spuC) and *E. coli* BL21 (pET-22b(+)4–Bm_AlaDh), respectively. The freshly-prepared strains were cultivated in 600 mL of the LB medium supplemented with 50 µg/mL antibiotic (ampicillin or kanamycin) in 1-L Erlenmeyer flasks at a rotary shaking rate of 220 rpm at 37°C. The recombinant protein expression was induced by adding isopropyl b-D-1-thiogalactopyranoside (IPTG) (0.2 mM, final) when A_{600} reached 0.6 - 0.8. The cell cultures were incubated at 18 °C for 16 h. The cells were harvested by centrifugation (4°C, 3,250xg, 20 min).

3.2 Protein purification

For purification purposes, cell pellets were resuspended (1g of wet cell paste/10 mL) in HEPES buffer (100mM, 1mM PLP, 5mM imidazole, pH 8). The cell pellets were lysed in an iced bath by ultra-sonication by Soniprep 150 (20 cycles of 20s on/20s off). After centrifugation (4^0C, 16,000xg, 20 min) the supernatant was used for protein purification manually or using an AKTA Pure system. Crude extract was then loaded onto a 5 mL HisTrap column. Purification was achieved manually or automated using an AKTA Pure system, using de-gassed HEPES buffer 1 (100 mM, 1mM PLP, 30mM imidazole, pH 8.0) and an elution buffer of HEPES buffer 2 (100 mM, 1mM PLP, 300mM imidazole, pH 8.0).

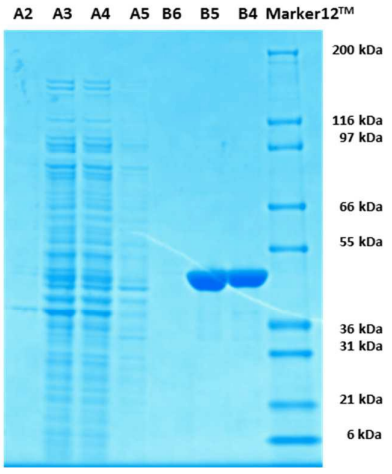

Figure S1. Representative SDS-PAGE analysis for the purification of the recombinant Pp-SpuC protein on AKTA (A2-A5 and B6: flow-through of crude extract during column loading; B5 and B4: fractions of Pp-SpuC protein post HisTag purification; Mark12™ Unstained Standard protein ladder).

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DOI: 10.1039/C6GC02102F

The column was initially washed with 5 column volumes HEPES buffer 1. The protein was then eluted with 10 column volumes of 100% elution buffer (50 mM HEPES, 1mM PLP, pH 9.0). 5 mL fractions were collected during the elution phase and a protein gel was run to identify fractions containing the transaminase protein, before these were pooled together. The protein solutions were concentrated in an Amicon® Ultra-15 10K centrifugal filter device and the purified enzyme was flash frozen and stored at –80°C. The purity was analysed by SDS/PAGE and the protein was more than 95% pure.

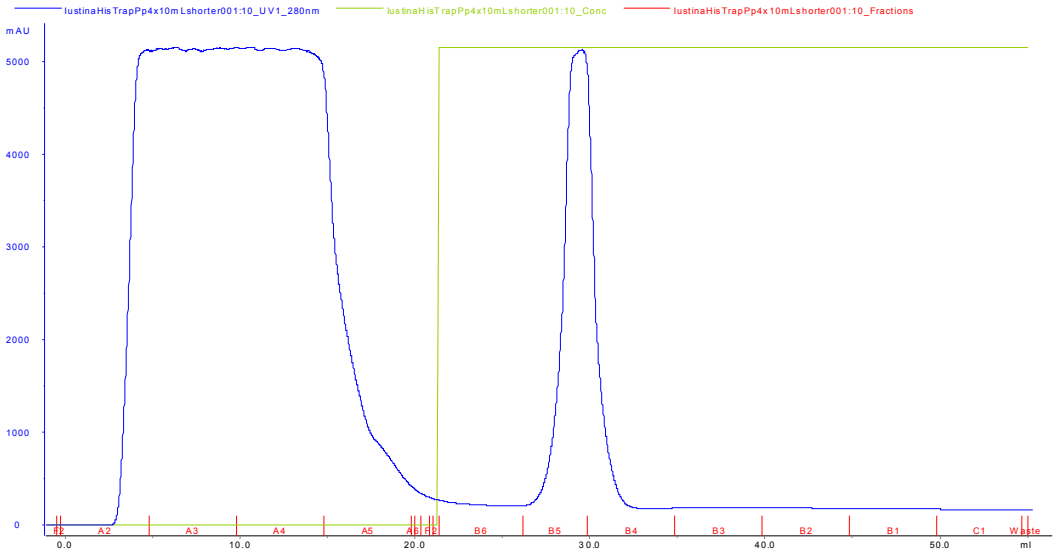

Figure S2. Representative chromatogram for purification of the His_6-tagged Pp-SpuC recombinant protein by metal ion affinity chromatography. Absorbance at 280 nm is shown in blue and the imidazole concentration of the buffer used in green (maximal value: 300 mM). The fractions collected (5mL each) are shown in red.

3.3 Protein determination and SDS-PAGE analysis

Concentrations of soluble protein was analysed by SDS-PAGE with 15% resolving gel and 5% stacking gel in a Tris-glycine buffer system. The gel was stained with Bio-Rad Coomassie Blue 250 kit (Bio-Rad lab, Munich, Germany), with bovine serum albumin (BSA) as a standard protein.

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DOI: 10.1039/C6GC02102F

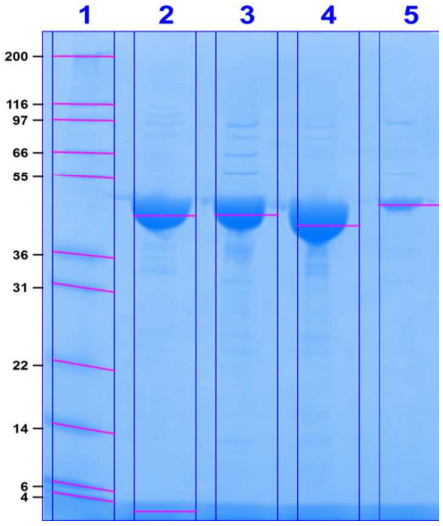

Figure S3. SDS-PAGE analysis of the three *spuC* recombinant proteins and Cv_TA (lane 1: *Marker 12* ; lane 2: *Pp_spuC*; lane 3: *Pf_spuC*; lane 4: *Pc_spuC*; lane 5: *Cv_TA)*

Table S3. Purification of recombinant *Pp_spuc*, *Pf_spuC* and *Pc_spuC* expressed in *E.coli*.

Recombinant protein	Culture medium volume (L)	Cell pellet (g)	Yield pure protein (g)	Purity (%)
Pp_spuC	0.6	5.8	0.200	98
Pf_spuC	0.6	6	0.250	94
Pc_spuC	0.6	5	0.390	95

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DOI: 10.1039/C6GC02102F

IV. Spectrophotometric activity analysis and determination of kinetic constants

4.1 L-Alanine dehydrogenase (L-AlaDh) activity assay screen

The transaminase activity was determined on a Tecan Sunrise™ plate reader, using a modified alanine dehydrogenase/ transaminase assay method. The spectrophotometric assay monitors the reduction of the tetrazolium salt INT by NADH, using 1-methoxy PMS as catalyst (λ= 503nm, ε= 5286.2 $M^{-1}cm^{-1}$).

Solution 1 (double-fold concentrated stock) was made up of the following: 2 mM nicotinamide adenine dinucleotide (NAD^+, 100 mM in water), 0.6 mM 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2*H*-tetrazolium (INT, 60 mM in DMSO), 0.01 mM 1-methoxy-5-methylphenazinium methyl sulfate (1-methoxy PMS, 1 mM in water), 0.05 mg/mL alanine dehydrogenase from *B. megaterium* (Bm_AlaDh) in 100 mM sodium phosphate buffer pH 8.

The 96-well substrate microplates contained 5 mM amine substrate (double-fold concentrated, in 100 mM sodium phosphate buffer, pH 8) and 0.2 mg/mL transaminase (double-fold concentrated purified enzyme in 100 mM HEPES buffer with 1 mM PLP, pH 8). 100 µL of solution 1 were added to the substrate microplate and the assay was initiated by addition of 2 mM sodium pyruvate (10 mM in water, double-fold concentrated).

Experiments were run on a spectrophotometer at 37 ºC with the measured activity at a wavelength of 503 nm and the absorbance taken every 13 seconds for 60 minutes. One Unit (U) is defined by one µmole of product per minute.

Specific activities against substrates **1a-k** are shown in Table S4.

Table S4. Specific activities for substrates **1a-k** determined using the L-AlaDh-TA assay.

	Specific activity U/mg				
substrate	Pp_spuC	Pf_spuC	Pc_spuC	Ec_yjgj	Cv_TA
1,4-diaminobutane	1.3	0.4	1.4	2.7	nd
1,5-diaminopentane	27.3	16.5	15.1	2.7	7.7
spermidine	15.7	16.5	21.5	2.1	nd
(S)-methylbenzylamine	23.2	25.7	30.4	nd	28.9
isopropylamine	20.4	18.5	18.7	0.05	5.6
butylamine	16.5	19.3	13.5	nd	14.1
1,6-diaminohexane	14.9	18.1	13.5	0.6	10.9
1,7-diaminoheptane	15.2	24.7	14.7	0.45	18.4
1,8-diaminooctane	7.5	2.7	2.3	0.33	4.4
1,9-diaminononane	13	4.4	3.1	nd	4.6
1,10-diaminodecane	2.1	3.8	1.7	nd	2.3

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4.2 Determination of kinetic parameters for substrates 1a-k

Kinetic parameters (Tables S5-S7) were deduced by non-linear least-square regression analysis based on Michaelis-Menten kinetics.

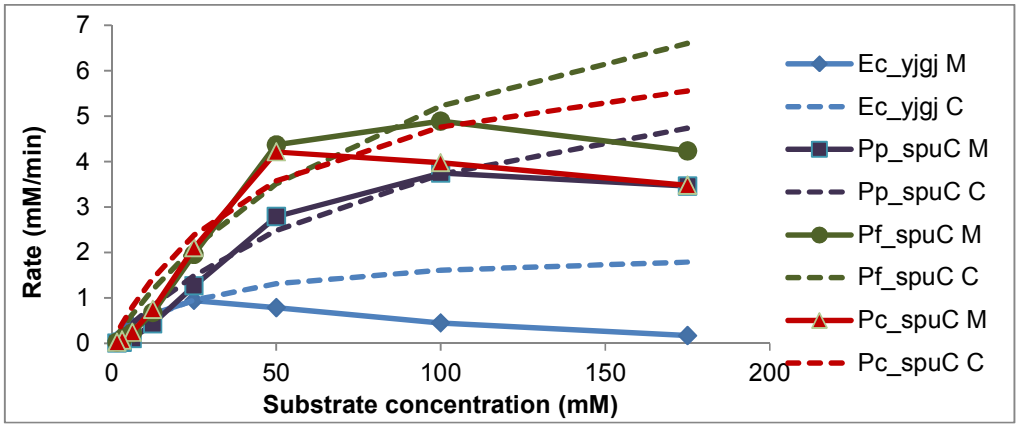

Figure S4. Determination of kinetic constants for Ec_ygjg, Pp_spuC, Pf_spuC and Pc_spuC for spermidine substrate using the L-Aladh/TA liquid phase assay. The measured (M, dotted line) and the calculated (C, solid line) rates of reactions for each protein are plotted against the substrate concentration. The kinetics parameters are determined using non-linear least-squares regression method.

Table S5. Values of Km for substrates **1a-k** determined using the L-AlaDh-TA assay.

	Km (mM)				
substrate	Pp_spuC	Pf_spuC	Pc_spuC	Ec_yjgj	Cv_TA
1,4-diaminobutane	62.34	52.99	59.87	0.47	Nd
1,5-diaminopentane	25	32.38	22.99	0.34	48.04
1,6-diaminohexane	1.41	2.31	4.05	2.54	3.92
1,7-diaminoheptane	1.06	1.02	0.64	0.44	1.09
1,8-diaminooctane	0.59	0.39	0.52	0.53	0.73
1,9-diaminononane	3.48	2.13	1.64	nd	2.11
1,10-diaminodecane	0.91	2.14	0.17	nd	0.34
spermidine	20.82	16.95	25.00	29.9	nd
(S)-methylbenzylamine	1.25	4.64	3.81	nd	1.06
butylamine	5.86	6.05	5.86	nd	10.19

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DOI: 10.1039/C6GC02102F

Table S6. Values of Vmax for substrates **1a-k** determined using the L-AlaDh-TA assay.

	Vmax (mM/min)				
substrate	Pp_spuC	Pf_spuC	Pc_spuC	Ec_yjgj	Cv_TA
1,4-diaminobutane	0.13	0.04	0.14	0.27	nd
1,5-diaminopentane	2.73	1.65	1.51	0.27	0.77
spermidine	1.57	1.65	2.15	2.1	nd
(S)-methylbenzylamine	2.32	2.57	3.04	nd	2.89
butylamine	1.65	1.93	1.35	nd	1.41
1,6-diaminohexane	1.49	1.81	1.35	0.06	1.09
1,7-diaminoheptane	1.52	2.47	1.47	0.05	1.84
1,8-diaminooctane	0.75	0.27	0.23	0.03	0.44
1,9-diaminononane	1.3	0.44	0.31	nd	0.46
1,10-diaminodecane	0.21	0.38	0.17	nd	0.23

Table S7. Values of Kcat for substrates **1a-k** determined using the L-AlaDh-TA assay.

	Kcat (s^{-1})				
substrate	Pp_spuC	Pf_spuC	Pc_spuC	Ec_yjgj	Cv_TA
1,4-diaminobutane	1.11	0.34	1.19	2.33	nd
1,5-diaminopentane	23.15	14.38	12.86	2.33	6.68
spermidine	13.32	14.38	18.31	18.17	nd
(S)-methylbenzylamine	19.68	22.41	25.89	nd	25.09
butylamine	13.99	16.82	1.50	nd	12.24
1,6-diaminohexane	12.64	15.77	11.49	0.51	9.46
1,7-diaminoheptane	12.89	21.53	12.51	0.38	15.97
1,8-diaminooctane	6.36	2.35	1.95	0.28	3.82
1,9-diaminononane	11.03	3.83	2.64	nd	3.99
1,10-diaminodecane	1.78	3.31	1.44	nd	1.99

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DOI: 10.1039/C6GC02102F

V. Biotransformations

5.1 Analytical scale

All biotransformations were carried out in 2 mL Eppendorf tubes, in a volume of 0.5 mL. To a mixture of amine substrate (25 mM from a 500 mM stock in 50 mM HEPES buffer, 1mM PLP, pH 9) and ketone (5 mM from a 500 mM stock solution in DMSO or MeOH), was added the enzyme (2mg/mL, purified or as cell-free lysate, prepared as described). The reactions were placed in a shaking incubator at 30°C and 250 rpm for 12 hours. The reactions were quenched by addition of 10 M NaOH (100µL), followed by extraction with methyl tert-butyl ether (300 µL). The organic phase was dried on $MgSO_4$ and analysed on normal phase chiral HPLC or GC-FID. For the GC-FID analysis, the samples were derivatized to the corresponding acetamides by addition of 30 µL Et_3N and 20 µL Ac_2O.

5.2 Preparative scale

In a 250 mL conical flask, the amine (20 mM, 2 eq) was dissolved in 50 mM HEPES buffer, containing 1mM PLP, pH 9 and the transaminase, Pp_spuC (60 mg from a stock of 20 mg/mL in buffer prepared as above) was added. The pH of the mixture was adjusted to 9 and the ketone (10 mM, 1eq) in DMSO (2.5% v/v) was added. The reaction was placed in a shaking incubator, 200 rpm, 30°C for 24h and the progress was followed by HPLC, with samples prepared as described in the analytical scale procedure. Samples were taken every hour for an initial period of 5 hours, followed by sampling at 19h, 21h and 29 h, respectively. The time course of the reaction is shown in Figure S5.

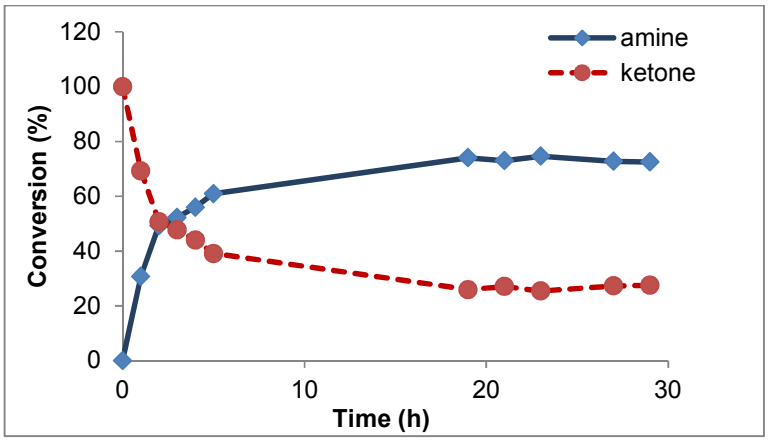
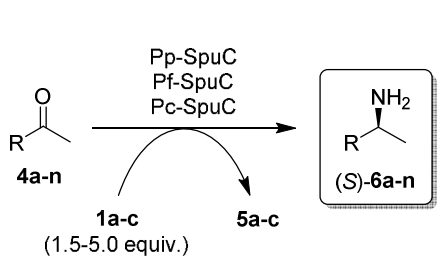
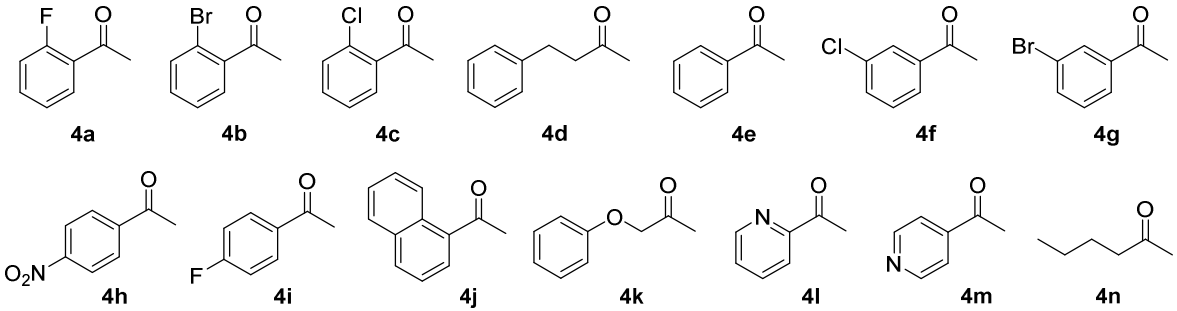


Figure S5. Kinetics of the transamination of 2-fluoroacetophenone (10 mM) by Pp_spuC, using cadaverine **1b** as amino donor (20 mM, 2 eq). The formation of the (*S*)-2-fluoromethylbenzylamine is shown in solid blue line, whilst the concentration of the 2-fluoroacetophenone is shown in red dotted line.

Table S8. Amination of ketones 4a-m with diamine donors **1a-c**.





	Diamine **1a**						Diamine **1b**						Diamine **1c**					
	Pp-SpuC		Pf-SpuC		Pc-SpuC		Pp-SpuC		Pf-SpuC		Pc-SpuC		Pp-SpuC		Pf-SpuC		Pc-SpuC	
Subs.	c (%)[a]	ee (%)[a]	c (%)[a]	ee (%)[a]	c (%)[a]	ee (%)[a]	c (%)[a]	ee (%)[a]	c (%)[a]	ee (%)[a]	c (%)[a]	ee (%)[a]	c (%)[a]	ee (%)[a]	c (%)[a]	ee (%)[a]	c (%)[a]	ee (%)[a]
4a	84	>99	92	>99	92[b]	>99	95	>99	95	>99	97	>99	83	>99	92	>99	97	>99
4b	80	>99	68	>99	89[b]	>99	68	>99	65	>99	93	>99	67	>99	65	>99	93	>99
4c	67	>99	75	>99	88[b]	96	88	>99	83	>99	91	>99	79	>99	81	>99	96	>99
4d	37[b]	69	34[b]	75	51[b]	9	64[b]	>99	59[b]	90	72	74	47	>99	41	50	90	36
4e	12[b]	>99	12[b]	>99	13[b]	82	42[b]	>99	25[b]	>99	34	>99	13	>99	13	>99	30	>99
4f	5[b]	>99	33[b]	>99	35[b]	>99	16[b]	>99	44[b]	>99	62	>99	8	>99	27	>99	74	>99
4g	63[b]	>99	5[b]	>99	85[b]	85	80[b]	>99	46[b]	>99	56	>99	62	>99	13	>99	86	>99
4h	41	>99	57	>99	99[b]	>99	59	>99	63	>99	93	>99	55	>99	63	>99	99	>99
4i	12	>99	11	>99	13[b]	94	28	80	13	>99	35	>99	13	>99	14	>99	37	93
4j	32[b]	>99	27[b]	>99	37[b]	>99	59[b]	>99	10[b]	>99	80	>99	28	>99	12	>99	38	>99
4k	92	>99	91	>99	35[b]	>99	99	96	98	>99	87	>99	88	56	81	>99	60	>99
4l	79	>99	77	>99	29[b]	83	96	>99	58	>99	5	>99	65	>99	91	>99	71	>99
4m	58	>99	49	>99	62[b]	>99	95	>99	73	>99	57	>99	55	>99	11	>99	44	>99

Expt. Cond.: 5 mM **4a-n**, 1.5 equiv. **1a-c**, 1mM PLP, 1% v/v DMSO, 2 mg mL^{-1} purified SpuC, 50 mM HEPES buffer, pH 9.0, 40°C, 250 rpm, 24 h.
a: determined by HPLC on a chiral stationary phase. b: in these cases 5. equiv. of amine donor were used to increase the conversion values.

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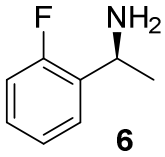
After 30 h, the reaction was stopped by addition of 10 M NaOH until the pH reached 12. The reaction was filtered through a short pad of Celite and extracted with MTBE (3 x 10 mL); the combined organics were washed with brine (1 x 20 mL) and dried on Na_2SO_4 and concentrated in vacuo to afford the amine as a pale yellow oil (55 mg).



6

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (td, *J* = 7.7, 2.0 Hz, 1H), 7.25 – 7.17 (m, 1H), 7.12 (td, *J* =7.5, 1.3 Hz, 1H), 7.01 (ddd, *J* = 10.9, 8.1, 1.3 Hz, 1H), 4.39 (q, *J* = 6.7 Hz, 1H), 1.42 (d, *J* = 6.7 Hz, 3H)

5.3 GC-assay conditions

GC-MS method: Hewlett Packard HP 6890 equipped with a HP-1MS column, a HP 5973 Mass Selective Detector and an ATLAS GL FOCUS sampling robot; method: 90^{0}C for 0 min, then 10^{0}C min^1 to 200C, hold for 10 min. GC FID method: Agilent 6850 equipped with a Gerstel Multipurposesampler MPS2L and an Agilent CHIRASIL-DEX CB 25 m x 0.25 mm DF=0.25 column; method: 120^{0}C isothermal, 15 min. The amines were analyzed by GC-FID as their corresponding acetamides, following derivatization as described previously.

5.4 HPLC assay conditions

Chiral normal phase HPLC was performed on an Agilent system (Santa Clara, CA, USA) equipped with a G1379A degasser, G1312A binary pump, a G1367A well plate autosampler unit, a G1316A temperature controlled column compartment and a G1315C diode array detector. CHIRALCEL® OD-H Analytical (Daicel, Osaka, Japan), 250 mm length, 4.6 mm diameter, 5 µm particle size) column was used. The typical injection volume was 15 µl and chromatograms were monitored at 265 nm. Solvent mixtures are given in *n*-hexane/isopropanol ratios (+0.1% diethylamine v/v).

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Table S9. Retention times for substrates and products

Ketone	Product	Amine	*n*-Hex/*i*-PrOH ratio
4a (4.59 min)	**6a**	(*R*)- 7.3 min, (*S*)- 7.81 min	95:5
4b (6.93 min)	**6b**	(*S*)- 9.83 min	95:5
4c (5.82 min)	**6c**	(*R*)- 7.3 min, (S)-9.07 min	95:5
4d (8.44 min)	**6d**	(*R*)- 13.01 min, (S)-18.28 min	95:5
4e (5.93 min)	**6e**	(*S*)- 11.09 min	95:5
4f (5.57 min)	**6f**	(*S*)- 9.41 mn	95:5
4g (5.81 min)	**6g**	(*S*)- 10.07 min	95:5
4h (11.37 min)	**6h**	(*S*)- 14.89 min	90:10
4i (5.53 min)	**6i**	(*S*)- 8.92 min	95:5
4j (9.05 min)	**6j**	(*S*)- 15.57 min	95:5
4k (10.5 min)	**6k**	(*R*)- 11.41 min, (S)- 17.87 min	90:10
4l (4.92 min)	**6l**	(*S*)- 8.3 min	90:10
4m (4.96 min)	**6m**	(*S*)- 11.45 min	90:10