Biocatalytic retrosynthesis approaches to D-(2,4,5-trifluorophenyl)alanine, key precursor of the antidiabetic sitagliptin

Fabio Parmeggiani,*[a] Arnau Rué Casamajo,[a] Danilo Colombo,[b] Maria Chiara Ghezzi,[b] James L. Galman,[a] Roberto A. Chica,[c] Elisabetta Brenna,[b] and Nicholas J. Turner*[a]

[a] Manchester Institute of Biotechnology (MIB), School of Chemistry, University of Manchester, 131 Princess Street, M1 7DN, Manchester, United Kingdom

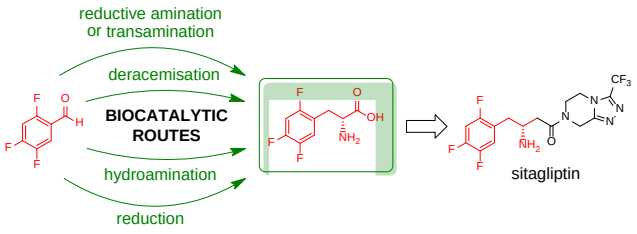
[b] Dipartimento di Chimica, Materiali e Ingegneria Chimica "G. Natta", Politecnico di Milano, Via Mancinelli 7, 20131, Milano, Italy

[c] Department of Chemistry and Biomolecular Sciences, Centre for Catalysis Research and Innovation, University of Ottawa, 10 Marie-Curie, Ottawa, Ontario, K1N 6N5, Canada

ABSTRACT

The integration of biocatalytic steps in retrosynthetic analysis of a target molecule offers multiple advantages, such as reduction of the environmental footprint of the process, viability of milder and safer reaction conditions, and accessibility of transformations that are challenging with traditional chemical synthesis. Herein, six chemo-enzymatic routes are described for the synthesis of a fluorinated D-phenylalanine derivative, precursor of the blockbuster antidiabetic drug sitagliptin. All routes start from the same aldehyde precursor and involve at least one biocatalytic step, including reductive amination, transamination, deracemisation, hydroamination, and alkene reduction. The target molecule was obtained in 2-5 steps from the aldehyde, with ee up to >99% and in 36-62% isolated yield. Furthermore, as part of one of the routes, the first example of a fully biocatalytic conversion of a cinnamic acid derivative to the corresponding D-phenylalanine (formal D-selective hydroamination) is reported.

GRAPHICAL ABSTRACT



KEYWORDS

biocatalysis, green chemistry, amino acids, type 2 diabetes, sitagliptin, enzymatic cascades

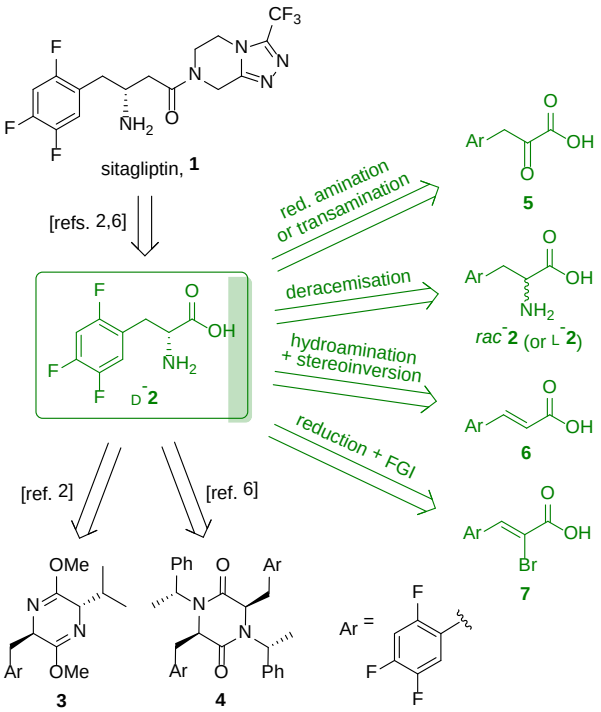
INTRODUCTION

Type 2 diabetes (T2D) is often referred to as a 'global epidemic' due to the worryingly high increase in the number of cases reported every year.[1] One of the most commonly employed antihyperglycemic drugs for the treatment of T2D is sitagliptin **1** (Scheme 1), first reported by Merck in 2005 as a potent, selective and orally active dipeptidyl peptidase IV (DPP-4) inhibitor.[2] Sitagliptin, in the form of phosphate salt monohydrate, was approved by the Food and Drug Administration of the U.S. in 2006 and rapidly turned into a blockbuster drug, either alone (Januvia®) or in combination with metformin (Janumet®). Nowadays, it is the most widely sold DPP-4 inhibitor worldwide, reaching sales of over 4 B$ in 2014 with an expected rise to >7 B$ in 2020.

The development of more efficient industrial syntheses of **1** is a compelling story[3] that enabled the discovery of new chemistries in the field of asymmetric catalysis and culminated with the widely acclaimed '3rd generation' chemoenzymatic route based on a transamination with a highly tailored biocatalyst by Merck and Codexis.[4] All the reported syntheses of **1** have been reviewed recently, highlighting the different stereoselective steps involved.[5]

The earliest synthesis published by Merck (as the original medicinal chemistry route) exploits D-(2,4,5-trifluorophenyl)alanine D-**2** (Scheme 1) as the key intermediate, converted to **1** by Arndt-Eistert homologation and amidation.[2] The amino acid D-**2** is obtained by hydrolysis of **3**, prepared by diastereoselective alkylation of the Schöllkopf diketopiperazine derivative of L-valine. This strategy has been revisited recently by Subbaiah and Haq, who instead prepared D-**2** from the hydrolysis of **4** (obtained by diastereoselective alkylation of the diketopiperazine derivative of (*R*)-1-phenylethylamine).[6] While reported yields from D-**2** to **1** are good (59% Merck, 69% Haq et al.), the preparation of D-**2** suffers from considerable disadvantages such as lower yields, (25% Merck, 59% Haq et al.), the need for an optically pure starting material (L-valine or (*R*)-1-phenylethylamine, respectively), and a low atom economy due to loss of the chiral auxiliary moiety.

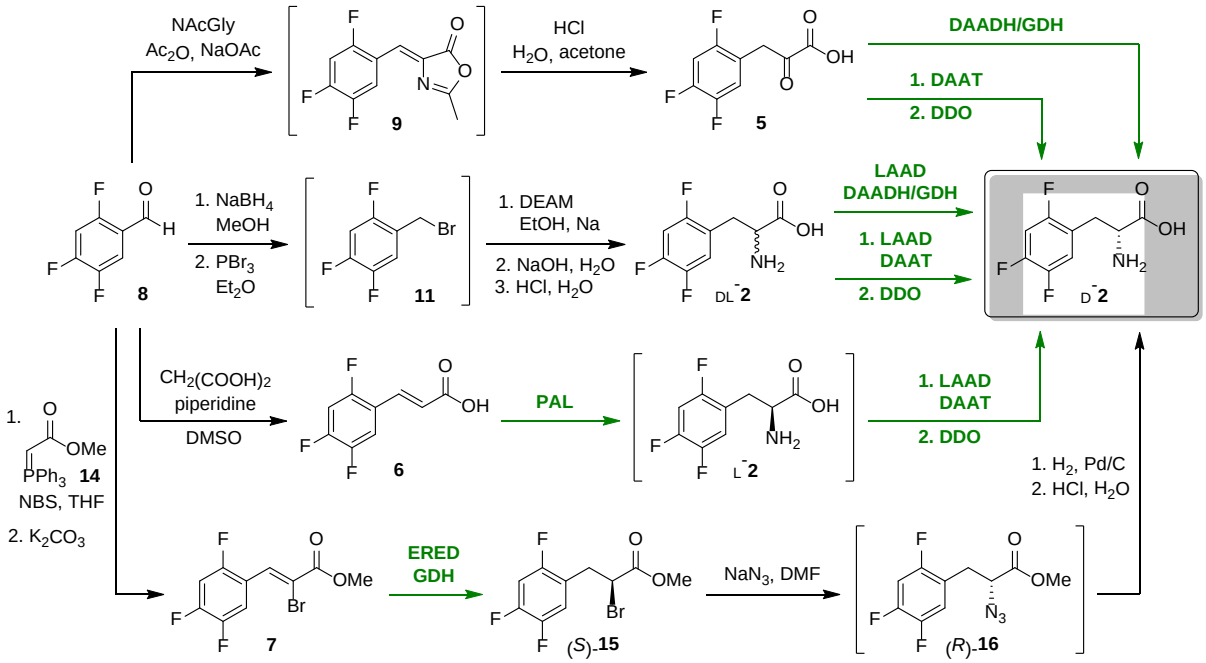
The replacement of chemical steps with biocatalytic reactions proved useful in countless case studies to improve the selectivity, yield or greenness of the entire process. In the past few years, we have been interested in biocatalytic syntheses of functionalised D-arylalanines, and herein we report on alternative approaches to D-**2**, in good yield, good to perfect enantioselectivity and in better accordance with the principles of green chemistry.



Scheme 1. Synthetic approaches to **1** based on D-**2** as the key intermediate.

RESULTS AND DISCUSSION

When performing a retrosynthetic analysis for the synthesis of new molecules, it can be very helpful for the organic chemist to consider alternative disconnections based on forward reactions enabled by known enzymes: this is the principle of 'biocatalytic retrosynthesis'.[7] For instance, in the case of the synthesis of D-**2**, we envisioned four possible alternative chemo-enzymatic processes (Scheme 1): reductive amination or transamination of the ketoacid **5**, deracemisation of racemic **2**, hydroamination of cinnamic acid **6** (that also requires a stereoinversion), and asymmetric reduction of bromoacrylate **7** with functional group interconversion (FGI) via azide. All the required starting materials were synthesised from the corresponding trifluorobenzaldehyde **8** (Scheme 2).



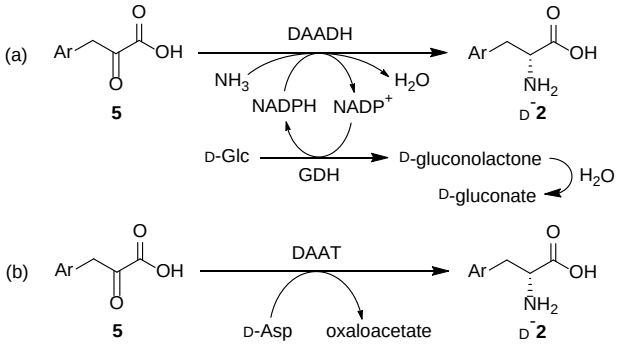
Scheme 2. General overview of the chemo-enzymatic routes from **8** to D-**2** considered in this work. Biocatalysed steps are highlighted in green.

Routes via ketoacid 5

Ketoacid **5** was prepared according to the standard Erlenmeyer-Plöchl synthesis via azlactone **9**. Aldehyde **8** was heated under reflux with *N*-acetylglycine (NAcGly), acetic anhydride, and anhydrous sodium acetate to yield a crude mixture of the azlactone **9** and a small amount of a partial hydrolysis product (the corresponding α-acetamidocinnamic acid). Without further purification, the mixture was subjected to complete hydrolysis with hydrochloric acid in water/acetone, yielding ketoacid **5** in 69% isolated yield after recrystallization (Scheme 1).

Two whole-cell biocatalytic reactions have been considered for the conversion of **5** to D-**2** (Table 1) a reductive amination mediated by an evolved D-amino acid dehydrogenase (DAADH)[8] and a transamination using an engineered D-amino acid transaminase (DAAT).[9] For the regeneration of the NADPH cofactor required by DAADH, a glucose dehydrogenase (GDH) was co-produced in the same host cell,[10] in the presence of D-glucose as an inexpensive sacrificial cosubstrate. The DAAT system, on the other hand, requires a single enzyme and a stoichiometric amount of D-aspartic acid as amino donor. Even though D-glutamic acid is a slightly better donor for DAAT, D-aspartic acid was used here because it can easily be removed by treatment with D-aspartate oxidase (DDO) after the reaction.[9]

Table 1. Screening of reductive amination (a) and transamination (b) of **5**.



Conc. 5 [mM]	CWW[a] [mg mL^{-1}]	(a) DAADH/GDH Conv. [%][b]	(b) DAAT Conv. [%][b]
10	25	>99	>99
25	50	>99	>99
50	50	>99	61
50	100	>99	85
100	100	71	56
100	150	66	64
150	150	15	19

Expt. cond.: (a) 4.0 equiv. D-Glc, 5.0 equiv. NH_4Cl, 1 mM $NADP^+$, 100 mM Na_2CO_3 buffer, pH 9.0, 37°C, 12 h; (b) 2.0 equiv. D-Asp, 1 mM PLP, 100 mM KP_i buffer, pH 8.0, 37°C, 12 h.
[a] CWW: cell wet weight.
[b] Determined by reverse-phase HPLC on a non-chiral phase.

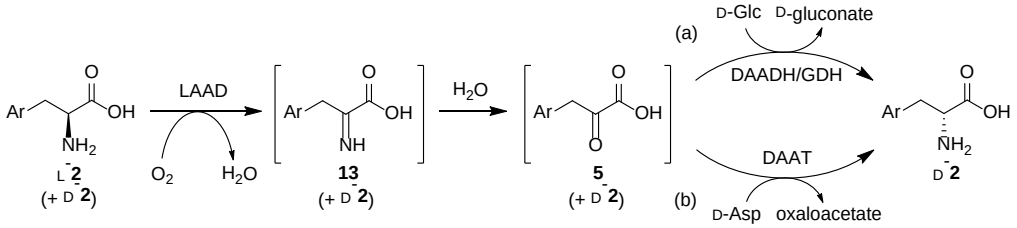
Both reactions worked very efficiently under standard conditions (Table 1), giving complete conversion after 12 h. In an attempt to improve the productivity of the overall process, higher substrate loadings were considered, obtaining good conversions up to 100 mM. Complete conversion was obtained for DAADH/GDH at up to 50 mM, while for DAAT this was achieved at up to 25 mM.
The typical procedure for recovery and purification of the amino acid product consists in adsorption on ion exchange resin (e.g., Dowex 50WX8) under acidic conditions, followed by elution with diluted ammonia solution. This technique is highly selective for amino acids and allows easy and rapid removal of buffers, salts and organic co-substrates. For the DAADH/GDH system, a preparative scale reaction with 50 mM **5** afforded 81% yield of D-**2** (>99% ee) after ion exchange. For the DAAT system, the product of a preparative reaction with 25 mM **5** was incubated with *E. coli* whole-cells producing bovine DDO (75 mg mL^{-1}) to oxidise excess aspartate, and then submitted to ion exchange purification, yielding 75% of isolated D-**2** (>99% ee). Therefore, it must be taken into account that the DAADH/GDH method requires a single whole-cell biocatalyst while the DAAT method requires two different strains in a two-step telescopic process.

Routes via racemic amino acid DL-2

Chemo-enzymatic deracemisation approaches, involving either a stereoselective step coupled to a non-selective chemical step, or two stereoselective steps, are very commonplace techniques to access enantiomerically pure compounds.[11] Regarding amino acid deracemisations in particular, it is a common strategy to couple oxidation of the L-enantiomer by L-amino acid deaminase (LAAD)[12] with a non-stereoselective chemical reduction, typically involving ammonia-borane.[13] Alternatively, this chemical reduction can be replaced by a second enzymatic step, resulting in a faster deracemisation rate (since both steps are stereoselective) and eliminating the need for overstoichiometric and expensive chemical reducing agents. For these reasons, it was decided to focus only on fully enzymatic deracemisation procedures.
The racemic phenylalanine DL-**2** was prepared by a multi-step sequence (Scheme 1). Firstly, **8** was reduced with sodium borohydride to the corresponding alcohol **10**, which was then converted to aryl bromide **11** with phosphorus tribromide. Both of these steps proceeded cleanly without side reactions, and the product did not require further purification. Although most published procedures for similar

brominations employ chlorinated solvents (typically CH_2Cl_2), in order to reduce the environmental impact of the process we instead performed the reaction in Et_2O. Then, alkylation of diethyl acetamidomalonate (DEAM) with **11** in the presence of sodium ethoxide in ethanol yielded the amidoester intermediate **12**, which gives DL-**2** by hydrolysis and decarboxylation. It is worth noting that complete hydrolysis of **12** to DL-**2** could not be achieved in acidic medium (aqueous conc. HCl), even after prolonged heating and in the presence of acetone, which was used as a co-solvent to increase the solubility of **12**. Therefore, a two-step hydrolysis procedure was employed, refluxing **12** in aqueous NaOH, followed by neutralisation and acidification with aqueous HCl and a second refluxing step.[14] At the end of the sequence, DL-**2** was crystallised from the hydrolysis mixture in 58% overall yield (from **8**).
The biocatalytic deracemisation of DL-**2** could be performed (Table 2) using whole-cell LAAD from *Proteus mirabilis* in combination with either of the two whole-cell systems described above (DAADH/GDH or DAAT). LAAD reacts stereoselectively with L-**2**, producing the imino acid **13**, which spontaneously hydrolyses to the ketoacid **5** that is a substrate of either DAADH (Table 2a) or DAAT (Table 2b). Complete deracemisation of up to 100 mM DL-**2** was achieved with 50 mg mL^{-1} of each whole-cell catalyst. However, it was not possible to reach very high ee values for D-**2** with higher substrate concentrations, even when increasing the whole-cell loading.

Table 2. Screening of biocatalytic deracemisations of DL-**2**.



	(a) LAAD-DAADH/GDH ee D [%][a]			(b) LAAD-DAAT ee D [%][a]		
Conc. DL-2 [mM]	**2 h**	**4 h**	**12 h**	**2 h**	**4 h**	**12 h**
25	>99	–	–	>99	–	–
50	54	96	>99	96	98	>99
100	37	56	88	85	94	>99
150	12	18	25	42	58	63

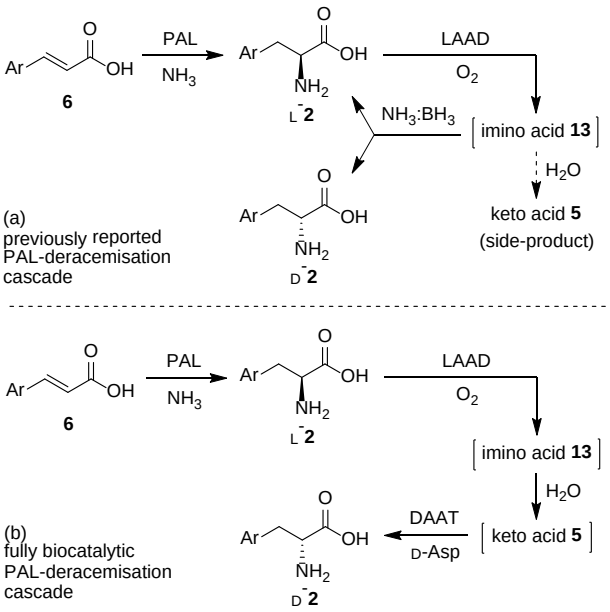
Expt. cond.: (a) 50-100 mg mL^{-1} LAAD whole-cells, 50-100 mg mL^{-1} DAADH/GDH whole-cells, 4.0 equiv. D-Glc, 5.0 equiv. NH_4Cl, 1 mM $NADP^+$, 100 mM Na_2CO_3 buffer, pH 9.0, 37°C; (b) 50-100 mg mL^{-1} LAAD whole-cells, 50-100 mg mL^{-1} DAAT whole-cells, 2.0 equiv. D-Asp, 1 mM PLP, 100 mM KP_i buffer, pH 8.0, 37°C.
[a] Determined by reverse-phase HPLC on a chiral phase.

Preparative reactions performed at 50 mM DL-**2** using the LAAD-DAADH/GDH system and at 100 mM DL-**2** using the LAAD-DAAT system afforded D-**2** in 79% and 74% yield after product isolation by ion exchange, respectively. Again, the LAAD-DAAT system also required an additional step of incubation of the crude reaction mixture with DDO prior to purification, to remove excess D-aspartate.

Route via cinnamic acid 6

Cinnamic acid **6** is an appealing starting substrate for enzymatic conversion since its synthesis from **8** can be achieved quantitatively in a single step (in contrast with **5** and DL-**2**, which are prepared using multi-step processes, in lower yields, and with the generation of larger amounts of waste). In a modification of the Knoevenagel-Doebner condensation, treatment of **8** with malonic acid and a catalytic amount of piperidine in DMSO (instead of the commonly employed and environmentally hazardous pyridine) at 85°C afforded **6** in high purity, without recrystallisation or purification (Scheme 1).
However, direct biocatalytic hydroamination of **6** to D-**2** is not possible using a single enzymatic reaction. Indeed, all known phenylalanine ammonia lyases (PALs) catalyse the enantioselective hydroamination of

cinnamic acids to yield L-phenylalanines[15] in high yield and almost perfect enantiopurity. In some cases, particularly with strong electron-withdrawing groups on the aromatic ring, lower ee values are observed,[16] but no useful enrichment in the D-enantiomer has been obtained as of yet. To address this issue, a one-pot conversion of cinnamic acids to D-amino acids was recently developed (Scheme 3a).[17] In this process, amination by PAL is combined with a stereoselective oxidation by LAAD and non-selective chemical reduction of the imino acid intermediate **13** with ammonia-borane complex. While this approach is efficient, leading rapidly to high ee values, it is not cost-effective because a large excess of reducing agent (40 equiv.) is required to reduce the imino acid before it hydrolyses to **5** (an observed side-product).

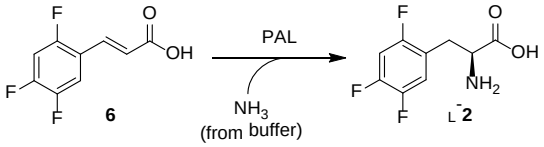


Scheme 3. Hydroamination-stereoinversion cascades for the synthesis of D-**2** from **6**: (a) previously reported system involving a chemical reduction; (b) novel fully biocatalytic alternative incorporating DAAT transamination.

A valuable alternative to this chemo-enzymatic process would be to let the hydrolysis of **13** proceed spontaneously to form **5**, and exploit one of the two biocatalytic transformations considered above (DAADH/GDH or DAAT, Table 1), to produce the D-amino acid. A preliminary test performed with unsubstituted L-phenylalanine demonstrated that the LAAD-DAADH/GDH system is strongly inhibited by the high ammonia concentrations required for the PAL reaction, but the LAAD-DAAT system is still effective, affording complete deracemisation in a few hours (ESI, Figure S1). Therefore, the only feasible combination would be the one shown in Scheme 3b.

Hydroamination of **6** was tested with different ammonia-containing buffers, with three whole-cell PAL biocatalysts: AvPAL from *Anabaena variabilis*,[18] RgPAL from *Rhodotorula glutinis*[19] and PbPAL from *Planctomyces brasiliensis*[20] (Table 3). Three common buffer systems were tested for this step (5 M ammonia solution adjusted to pH 9.6 with sulphuric acid, 5 M ammonia solution adjusted to pH 10.0 with dry ice, and 4 M unadjusted ammonium carbamate solution). Additionally, since high ammonia concentrations are detrimental to the enzymes involved in the deracemisation, the reaction was also tested with the same buffer systems with halved ammonia concentration. The reaction proceeded in excellent yields with all three PALs, affording L-**2** in 61-99% enantiomeric purities (see ESI, Table S1). The lower ee values obtained for several of these reactions can be ascribed to the presence of three fluorine atoms on the phenyl ring, which reduce electron density of the double bond, as previously reported.[16-17] It is worth noting that while decreased ammonia concentrations severely affected the performance of RgPAL (e.g., from 97% to 42%), a much less dramatic effect was observed for AvPAL and PbPAL (e.g. 91-99% to 78-82%, with carbamate).

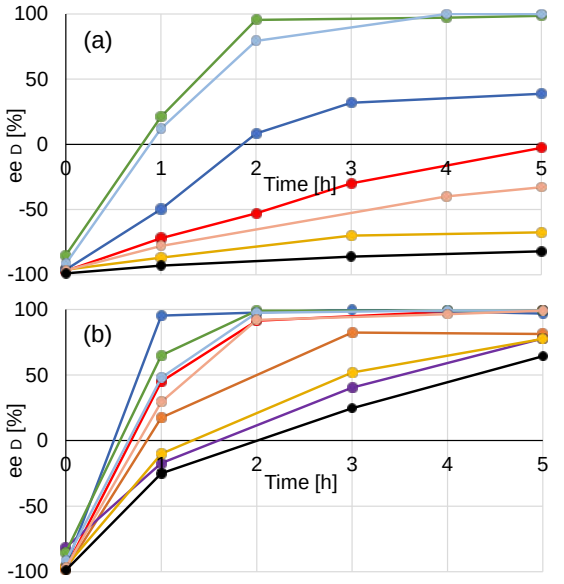
Table 3. Screening of PAL-mediated hydroamination of **6**.



Buffer	AvPAL Conv. [%][a]	RgPAL Conv. [%][a]	PbPAL Conv. [%][a]
NH_3/H_2SO_4 5 M, pH 9.6	84	63	83
NH_3/H_2SO_4 2.5 M, pH 9.6	68	21	71
NH_3/CO_2 5 M, pH 10.0	97	41	92
NH_3/CO_2 2.5 M, pH 10.0	85	34	82
$H_2NCOONH_4$ 4 M, pH 9.9	99	97	91
$H_2NCOONH_4$ 2 M, pH 9.9	78	42	82

Expt. cond.: 50 mM **6**, 50 mg mL^{-1} PAL whole-cells, 37°C, 12 h.
[a] Determined by reverse-phase HPLC on a non-chiral phase.

To identify the best conditions to perform the stereoinversion of L-**2** to D-**2** following hydroamination, cells producing DAAT and excess D-aspartate were added to the highest-yielding PAL reactions shown in Table 3 (with different biocatalysts or buffers). Analogous reactions with the same buffers but with different substrate loadings were also tested. The ee values were monitored over time (Figure 1a, only the runs that showed an >80% overall conversion of **6** to D-**2** are reported).



Subs. [mM]	Buffer	PAL	Conv. 6 [%]
25	5 M NH_3/H_2SO_4	AvPAL	97
25	5 M NH_3/CO_2	AvPAL	98
50	5 M NH_3/H_2SO_4	AvPAL	84
50	5 M NH_3/CO_2	AvPAL	97
50	5 M NH_3/CO_2	PbPAL	92
50	4 M $H_2NCOONH_4$	PbPAL	91
50	2.5 M NH_3/CO_2	AvPAL	85
50	2 M $H_2NCOONH_4$	PbPAL	82
100	5 M NH_3/CO_2	AvPAL	91

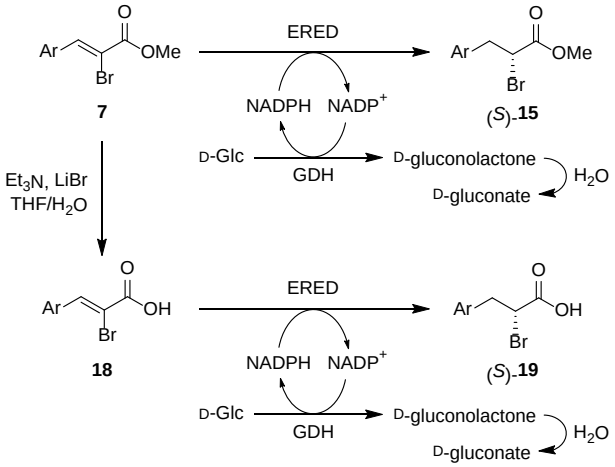
Figure 1. Screening of the stereoinversion of L-**2** as a one-pot system following the hydroamination of **6**. (a) deracemisations performed directly on the PAL amination reaction mixtures; (b) deracemisations performed on the PAL amination reaction mixtures after dilution with an equal volume of water.

Remarkably, complete stereoinversion was observed for 25 mM **6** with AvPAL in 5 M NH_3/H_2SO_4 buffer and for 50 mM **6** with PbPAL in 2 M carbamate. However, the stereoinversion rates dropped considerably with increased substrate and ammonia concentration, as anticipated. Therefore, the same stereoinversion reactions were also tested under identical conditions and with the same biocatalyst loadings, but diluting the PAL reaction mixture with an equal volume of water before the addition of LAAD and DAAT cells (Figure 1b). In all cases the reaction proceeded much faster, reaching very high ee values within 1 or 2 hours, and multiple tests afforded almost complete deracemisation up to 50 mM **6** (Figure 1b). For higher substrate concentrations (100 mM) satisfactory ee values could not be obtained, even with longer incubation times. The reduction of ammonia concentration by dilution in between the two steps allows a convenient trade-off to be reached between the conversion of **6** (>80%) and the deracemisation rate of **2** (>95% ee) up to 50 mM **6**.
A preparative scale biotransformation with 50 mM **6** under optimal conditions (incubation with AvPAL in 2.5 M NH_3/CO_2 buffer, followed by dilution and incubation with LAAD and DAAT) gave complete deracemisation in a few hours. For product isolation, the same method described above for the DAAT reactions was applied, i.e. treatment with DDO and ion exchange purification, affording D-**2** in 67% yield from **6**.

Route via bromoacrylate 7

Another route to the D-amino acid D-**2** was designed by exploiting the asymmetric hydrogenation capability of ene-reductases (EREDs), also known as Old Yellow Enzymes (OYEs).[21] These flavin-dependent oxidoreductases are known to reduce C=C double bonds activated by one or more electron-withdrawing groups such as carbonyl or nitro groups. The reduction of α-haloacrylates by EREDs is a well-known example of efficient bioreduction of substrates activated by only one poorly activating electron-withdrawing groups (the methyl ester moiety).[22]
Bromoacrylate **7** (Scheme 1) could be prepared via a modified Horner-Wadsworth-Emmons condensation based on the generation of a stabilised haloylide by bromination of **14** with NBS, followed by condensation with **8** in the presence of potassium carbonate.[23] This one-pot procedure affords **7** in 76% isolated yield, as a 96:4 *Z*/*E* mixture. Recrystallisation from *n*-hexane afforded pure (*Z*)-**7** in >99% diastereomeric excess. The reduction of (*Z*)-**7** to the saturated bromoester (*S*)-**15** was tested with a panel of ERED biocatalysts, using again the GDH/glucose regeneration system for the NADPH cofactor (Table 4). Interestingly, very different conversions were observed with different members of the OYE family, the best being OYE3 from *Saccharomyces cerevisiae*, which afforded 78% conversion and perfect enantioselectivity. Using directly the 96:4 *Z*/*E* mixture as a substrate afforded only lower ee values (≤90%, ESI, Table S2), which is consistent with previous reports regarding this class of haloesters, where the two stereoisomers were found to give the opposite enantiomer of the product with OYE1-3.[22b] Additionally, bromoacrylic acid **18** was synthesised by hydrolysis of **7** and tested against the same panel of EREDs (Table 4). However, no conversion could be observed, presumably due to the insufficient electron-withdrawing ability of the carboxylate group.

Table 4. Preliminary screening of a panel of EREDs for the reduction of **7** and **18**.



ERED	Conv. 7 [%][a]	ee (*S*)-15 [%][b]	Conv. 18 [%][a]
OYE1	55	95	<1
OYE2	13	n.d.[c]	<1
OYE3	78	99	<1
OYE2.6	53	57	<1
YqjM	<1	–	<1
NemA	<1	–	<1
LeOPR1	<1	–	<1
PETNR	<1	–	<1

Expt. cond.: 5 mM **7**, 0.1 mg mL^{-1} isol. ERED, 0.05 mg mL^{-1} isol. GDH, 4.0 equiv. D-Glc, 0.1 mM NADP$^+$, 5% *v/v* DMSO, 50 mM KP$_i$ buffer, pH 7.0, 30°C, 24 h. [a] Determined by GC-MS. [b] Determined by direct-phase HPLC on a chiral phase. [c] Not determined.

In order to minimise the cost of the biocatalytic step, the reduction of **7** was tested as a whole-cell system using *E. coli* cells overproducing OYE3 instead of the isolated enzyme. Unfortunately, only very low conversions were observed (data not shown). As an alternative, the use of raw cell lysate was also investigated, also testing higher substrate concentrations in an attempt to improve the productivity of the process (ESI, Table S3).

A preparative scale biotransformation (110 mg **7**) performed under optimised conditions, followed by a simple extraction with EtOAc afforded (*S*)-**15** in 84% yield, >99% ee and sufficient purity for further processing. Unlike the other routes discussed above, in this case the biotransformation product (*S*)-**15** needs to be further manipulated to afford D-**2**. Therefore, a three-step FGI procedure was performed, consisting of nucleophilic substitution with sodium azide in DMF to form the azido ester (*R*)-**16** with inversion of configuration, catalytic hydrogenation to form amino ester (*R*)-**17** and hydrolysis with concentrated HCl to afford D-**2** (Scheme 1). The three reactions could be carried out sequentially with quantitative conversion and no need for chromatographic purification of any of the intermediates. The product was recovered from the aqueous mixture with the same ion exchange procedure described above, in 81% yield from (*S*)-**15**. The optical purity of intermediates **16** and **17** could not be verified with the analytical tools available, and the D-**2** recovered showed an ee value of 96%. It is worth noting that in a preliminary attempt to replace DMF with acetone in the nucleophilic substitution step (from (*S*)-**15** to (*R*)-**16**), the final amino acid product was recovered with very low enantiopurity (<20% ee). Therefore, the partial loss of optical purity likely took place during the substitution step.

Comparison of the proposed routes

The selection of the best route towards a particular synthetic target is often more an art than a science. Many aspects that frequently play against each other must be taken into account, and consequently different routes could be ranked differently in different contexts. In this case, more than trying to identify the best method, it was our purpose to show and compare the strengths and weaknesses of each, as summarised in Table 5. The parameters considered for the comparison of the "efficiency" of the route are the overall yield (from **8** to D-**2**), the enantiopurity of the product, the number of steps required, and the substrate concentration of the biocatalytic step. The latter parameter was chosen because the biotransformation is often the bottleneck that limits the productivity of the process. The overall space-time yield was not considered because reaction times were not optimised (in particular for enzymatic reactions performed with 12-24 h incubation).

Regarding the environmental impact, a detailed calculation of the environmental factor (E-factor)[24] would require thorough optimisation of reaction conditions and complete understanding of solvent recyclability, which is rarely attempted in early-stage development or at route selection. Instead, we estimated the simplified E-factor (sEF) according to Roschangar *et al.*,[25] which does not take into account water (considered non-hazardous) and all recyclable solvents (assuming complete recovery). This assumption is convenient because the use of such materials has not been individually optimised, and including their amounts in a rigorous E-factor calculation would be unfair and not representative. Calculated sEF values are reported in Table 5 (see ESI for the details). It should be noted that, due to its definition, this metric should only be used for the purpose of comparison, and not to draw conclusions on the actual amount of waste produced.

Table 5. Summary and comparison of the alternative chemoenzymatic routes to D-**2**.

Route	**Overall yield from 8 [%]**	**ee D-2 [%]**	**Number of steps**	**Subs. loading [g L^{-1}][a]**	**Simplified E-factor (sEF)[b]**
Reductive amination of **5**	56	>99	3	10.9	~21
Transamination of **5**	52	>99	3	5.4	~30
Deracemisation of DL-**2** (DAADH)	46	>99	5	10.9	~41
Deracemisation of DL-**2** (DAAT)	43	>99	5	21.9	~41
Hydroamination of **6** + stereoinversion	62	>99	2	10.1	~65
Reduction of **7** + FGI	36	96	5	5.5	~82

[a] Substrate concentration for the biocatalytic step. [b] Not including water and recyclable solvents, as described in ref. 25 (see ESI for detailed calculation).

All the routes presented are based on at least one biocatalytic step, which proceeds under mild conditions in an aqueous environment. Only one chromatographic separation (purification of **7**) was required, all the environmentally unfriendly solvents were eliminated or replaced with more benign alternatives, and no heavy metals were employed throughout. These considerations are of paramount importance in order to improve and simplify pharmaceutical and fine chemical manufacturing, according to the principles of green chemistry.

Each method shows different advantages and disadvantages, and could become more favourable under certain circumstances, depending on other constraints. However, the hydroamination-stereoinversion route is perhaps preferable in terms of overall yield and simplicity of the process, even though the estimated waste generation is not the lowest. Furthermore, this biocatalytic system shows considerable advantages over the previously reported hydroamination-stereoinversion cascade: it is entirely enzyme catalysed, it does not require unstable or hazardous reducing agents in large excess, and it proceeds more rapidly since it incorporates two stereoselective steps in the stereoinversion (as opposed to one selective and one non-selective as in previous chemo-enzymatic deracemisation processes).

CONCLUSION

The development of alternative synthetic routes to established pharmaceutical molecules is not only crucial to improve the yields and minimise the environmental impact of known routes, but it can also be useful to circumvent IP-protected reactions and procedures, for instance in the industry of generic pharmaceuticals.
Here, we described a variety of biocatalytic and chemo-enzymatic procedures to access a key D-amino acid intermediate for the production of the blockbuster antidiabetic sitagliptin. The procedures are intrinsically environmentally friendly and have been demonstrated on preparative scale, with increased substrate loadings compared to the usual screening conditions for new biocatalytic reactions (1-5 mM), in order to maximise the productivity of the process.
Of note, we developed a new one-pot fully biocatalytic route from cinnamic acids to D-phenylalanines. This system includes the transamination of the ketoacid instead of a chemical reduction of the imino acid used in previously reported processes, eliminating the need for chemical reducing reagents in large excess. In terms of operational simplicity and environmental impact this procedure seems to be the most promising of the routes reported in this work.
Furthermore, the same procedures and protocols could in principle be transferred to other D-arylalanines, which are common intermediates for various APIs, opening up a range of new synthetic strategies based on the idea of biocatalytic retrosynthesis.

CONFLICTS OF INTEREST

There are no conflicts of interest to declare

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EXPERIMENTAL SECTION

Chemical synthesis

2-Oxo-3-(2,4,5-trifluorophenyl)propanoic acid (5)
Aldehyde **8** (2.50 g, 15.6 mmol), *N*-acetylglycine (2.55 g, 21.8 mmol, 1.4 equiv.) and anhydrous NaOAc (1.78 g, 21.8 mmol, 1.4 equiv.) were added to Ac_2O (15 mL) and the mixture was stirred and heated at 110°C under reflux for 6 h. The solution was cooled in an ice bath and the precipitated yellow solid was filtered and washed with ice-cold water (2 × 30 mL). This solid (shown by ^{1}H NMR to be a mixture of the azlactone **9** and the corresponding α-acetamidocinnamic acid in ~9:1 ratio) could not be purified further by recrystallisation from EtOH/H_2O and was used directly for the hydrolysis step. The solid was suspended in a mixture of aqueous HCl (25 mL, 3 M) and acetone (5 mL). The slurry was heated under reflux for 6 h, after which the hydrolysis was complete (by ^{1}H NMR). The solvent was partially removed under reduced pressure, causing the crystallisation of the ketoacid. Cream coloured solid, yield 2.36 g (69%). ^{1}H NMR (400 MHz, CD_3OD): δ 8.2 (ddd, J = 16.4, 9.4, 7.1 Hz, 1H), 6.94 (dd, J = 10.4, 6.9 Hz, 1H), 6.55 (s, 1H). ^{13}C NMR (101 MHz, CD_3OD): δ 167.3, 156.5 (ddd, J = 248.1, 8.7, 2.2 Hz), 150.0 (ddd, J = 250.5, 14.1, 12.3 Hz), 147.8 (ddd, J = 240.8, 12.7, 3.6 Hz), 144.5, 121.1 (ddd, J = 13.9, 7.5, 4.4 Hz), 118.9 (dd, J = 21.6, 3.9 Hz), 105.9 (dd, J = 29.1, 21.4 Hz), 99.6 (d, J = 7.1 Hz). ^{19}F NMR (376 MHz, CD_3OD): δ –120.6 (dd, J = 14.8, 3.1 Hz), –136.8 (dd, J = 21.3, 3.1 Hz), –146.3 (dd, J = 21.3, 14.8 Hz).

1-(Bromomethyl)-2,4,5-trifluorobenzene (11)
Aldehyde **8** (5.00 g, 31.2 mmol, 1.0 equiv.) was dissolved in MeOH (80 mL) and cooled in an ice bath. $NaBH_4$ (0.59 g, 15.6 mmol, 0.5 equiv.) was added portion-wise under stirring. At the end of the addition the ice bath was removed and the mixture was stirred at ambient temperature for 3 h, after which the conversion was complete (by GC-MS). The mixture was quenched with ice-cold water (10 mL) and most of the solvent was evaporated under reduced pressure. The residue was diluted with brine (20 mL) and extracted with Et_2O (3 × 25 mL). The combined organic phase was washed with brine (20 mL) and dried over anhydrous $MgSO_4$ to yield a solution of (2,4,5-trifluorophenyl)methanol (**10**) to be employed in the next step. A small sample was evaporated for characterisation, yielding a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.30-7.21 (m, 1H), 6.94-6.85 (m, 1H), 4.67 (s, 2H), 2.25 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 155.1 (ddd, J = 244.9, 9.5, 2.7 Hz), 149.4 (ddd, J = 250.8, 14.6, 12.5 Hz), 146.9 (ddd, J = 240.8, 12.6, 3.7 Hz), 124.2 (ddd, J = 17.0, 4.9, 4.2 Hz), 116.8 (ddd, J = 19.7, 5.9, 1.2 Hz), 105.4 (dd, J = 27.3, 20.9 Hz), 58.1 (d, J = 3.8 Hz). ^{19}F NMR (376 MHz, $CDCl_3$): δ –121.4 (dd, J = 15.6, 3.4 Hz), –134.9 (dd, J = 21.4, 3.3 Hz), –142.7 (dd, J = 21.4, 15.6 Hz). GC-MS (EI): t_R = 5.12 min, *m/z* (%) = 162 (100, M^+), 141 (77, M^+), 134 (70), 113 (64). The solution was cooled in an ice bath and PBr_3 (10.1 g, 37.4 mmol, 1.2 equiv.) was added dropwise under stirring. The ice bath was removed and the mixture was stirred for 4 h at ambient temperature. Ice-cold water (50 mL) was added, the organic layer was separated and the aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic phase was washed with aq. $NaHCO_3$ (30 mL, 10%) and brine (30 mL), dried over anhydrous $MgSO_4$ and evaporated under reduced pressure, yielding the aryl bromide **11**, used without further purification. Colourless oil, yield 5.54 g (79%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.27-7.19 (m, 1H), 6.94 (dd, J = 9.5, 6.5 Hz, 1H), 4.43 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 155.9 (ddd, J = 248.9, 9.5, 2.9 Hz), 150.4 (ddd, J = 253.3, 14.6, 12.3 Hz), 146.9 (ddd, J = 246.4, 12.6, 3.8 Hz), 121.7 (ddd, J = 17.0, 4.9, 4.2 Hz), 118.9 (ddd, J = 19.7, 4.5, 1.7 Hz), 106.1 (dd, J = 27.4, 21.2 Hz), 24.2 (d, J = 3.8 Hz). ^{19}F NMR (376 MHz, $CDCl_3$): δ –118.0 (dd, J = 15.4, 4.7 Hz), –131.9 (dd, J = 21.1, 4.7 Hz), –142.7 (dd, J = 21.1, 15.4 Hz). GC-MS (EI): t_R = 5.28 min, *m/z* (%) = 226 (5, M^+), 224 (5, M^+), 145 (100), 125 (15).

Diethyl 2-acetamido-2-(2,4,5-trifluorobenzyl)malonate (12)
Sodium metal (0.36 g, 15.5 mmol, 1.1 equiv.) was dissolved in abs. EtOH (30 mL) at r.t., then DEAM (3.17 g, 15.5 mmol, 1.1 equiv.) was added under vigorous stirring. After 10 min, crude **11** (3.37 g, 14.1 mmol, 1.0 equiv.) was added portionwise and the mixture was heated and stirred under reflux for 6 h. The white slurry was concentrated under reduced pressure to almost complete dryness and recrystallysed from boiling EtOH/H_2O (7:3). White crystals, yield 4.28 g (84%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.90-6.81 (m, 2H), 6.55 (s, 1H), 4.32-4.18 (m, 4H), 3.65 (s, 2H), 2.00 (s, 3H), 1.28, (t, J = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 169.5, 167.4, 156.6 (ddd, J = 245.0, 9.2, 2.3 Hz), 149.3 (ddd, J = 251.2, 14.3, 12.8 Hz), 146.6 (ddd, J = 245.0, 12.5, 3.7 Hz), 119.9 (ddd, J = 19.3, 6.0, 1.0 Hz), 118.8 (ddd, J = 18.8, 5.9, 4.6 Hz), 105.5 (dd, J = 28.9, 20.7 Hz), 66.1, 63.0, 31.4, 23.0, 14.0. ^{19}F NMR (376 MHz, $CDCl_3$): δ –118.2 (dd, J = 15.4, 3.5 Hz), –134.4 (dd, J = 21.6, 3.5 Hz), –142.9 (dd, J = 21.6, 15.4 Hz). HRMS (ESI): *m/z* for $[M+H]^+$ $C_{16}H_{19}F_3NO_5^+$ calcd. 362.1210, found 362.12206.

DL-2-amino-3-(2,4,5-trifluorophenyl)propanoic acid (DL-2)
Amidoester **12** (4.28 g, 11.8 mmol) was suspended in MeOH (50 mL). A solution of NaOH (4.72 g, 118 mmol) in water (12 mL) was added while stirring and the mixture was refluxed for 3 h. The solution was acidified with conc. HCl (15 mL), then most of the MeOH was removed under reduced pressure. Additional conc. HCl (15 mL) was added and the mixture was refluxed again for 3 h, yielding a completely clear solution. The pH was adjusted to neutral with conc. aqueous NH_3, the solution was cooled to –20°C until crystallisation occurred and the product was recovered by filtration under reduced pressure. White crystals, yield 2.24 g (87%). ^{1}H NMR (500 MHz, D_2O+NaOH): δ 7.09 (ddd, J = 11.0, 9.0, 6.9 Hz, 1H), 7.00 (ddd, J = 10.6, 9.7, 6.8, 1H), 3.38 (dd, J = 7.1, 6.3 Hz, 1H), 2.85 (dd, J = 13.9, 6.3 Hz, 1H), 2.74 (dd, J = 13.9, 7.5 Hz, 1H). ^{13}C NMR (126 MHz, D_2O+NaOH): δ 181.6, 156.2 (ddd, J = 242.0, 9.6, 2.3 Hz), 148.5 (ddd, J = 246.5, 14.4, 13.0 Hz), 146.3 (ddd, J = 241.2, 12.4, 3.6 Hz) 121.5 (ddd, J = 18.9, 5.8, 4.1 Hz), 118.9 (dd, J = 19.1, 6.3 Hz), 105.2 (dd, J = 29,1, 21.0 Hz), 56.5, 33.6. ^{19}F NMR (470 MHz, D_2O+NaOH): δ –119.6 (dd, J = 15.5, 3.4 Hz), –137.2 (dd, J = 21.8, 3.4 Hz), –144.3 (dd, J = 21.8, 15.5 Hz). HRMS (ESI): *m/z* for $[M+H]^+$ $C_9H_9F_3NO_2^+$ calcd. 220.0580, found 220.05775.

(*E*)-3-(2,4,5-trifluorophenyl)acrylic acid (6)
Aldehyde **8** (2.50 g, 15.6 mmol), malonic acid (4.87 g, 46.8 mmol, 3 equiv.) and piperidine (0.5 mL) were added to DMSO (30 mL) and the mixture was stirred under reflux for 4 h. The solution was cooled to ambient temperature and poured into ice-cold aqueous HCl (100 mL, 3 M). The precipitate was filtered and washed with water (2 × 30 mL) and aqueous $NaHCO_3$ (10 mL, 10%). The solid was dried and used without further purification. White solid, yield 2.89 g (92%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.11-7.99 (m, 1H), 7.68-7.57 (m, 1H), 7.53 (d, J = 16.4 Hz, 1H), 6.63 (d, J = 16.4 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 167.1, 156.0 (ddd, J = 250.4, 10.0, 2.1 Hz), 150.2 (dt, J = 252.6, 13.8 Hz), 146.5 (ddd, J = 242.8, 12.5, 2.9 Hz), 133.6 (d, J = 2.5 Hz), 122.9 (dd, J = 4.6, 2.3 Hz), 119.1 (ddd, J = 13.8, 6.6, 4.3 Hz), 116.6 (dd, J = 20.1, 3.8 Hz), 106.6 (dd, J = 29.1, 21.6 Hz). 156.2 (ddd, J = 242.0, 9.6, 2.3 Hz), 148.5 (ddd, J = 246.5, 14.4, 13.0 Hz), 146.3 (ddd, J = 241.2, 12.4, 3.6 Hz) 121.5 (ddd, J = 18.9, 5.8, 4.1 Hz), 118.9 (dd, J = 19.1, 6.3 Hz), 105.2 (dd, J = 29,1, 21.0 Hz), 56.5, 33.6. ^{19}F NMR (376 MHz, DMSO-d_6): δ –116.9 (dd, J = 15.5, 5.6 Hz), –130.7 (dd, J = 22.8, 5.6 Hz), –142.2 (dd, J = 22.8, 15.5 Hz). HRMS (ESI): *m/z* for $[M+H]^+$ $C_9H_6F_3O_2^+$ calcd. 203.0314, found 203.03103.

(*Z*)-methyl 2-bromo-3-(2,4,5-trifluorophenyl)acrylate (7)
NBS (3.92 g, 22 mmol, 1.1 equiv.) was added portionwise under N_2 atmosphere to a solution of methyl(triphenylphosphoranylidene) acetate **14** (6.68 g, 20 mmol, 1.0 equiv.) in THF (60 mL) at –20 °C. The mixture was stirred at –20 °C for 1 h and then warmed to room temperature. Aldehyde **8** (3.20 g, 20 mmol) and K_2CO_3 (6.90 g, 50 mmol, 2.5 equiv.) were added to the mixture, which was stirred for 16 h. The reaction mixture was poured into water (60 mL) and extracted with EtOAc (3 × 20 mL). The organic phase was dried with Na_2SO_4 and the solvent was removed under reduced pressure to give a residue, which was purified by silica gel column chromatography (*n*-hexane/EtOAc). White solid, yield 4.48 g (76%), de 92% (by ^{1}H NMR). Recrystallisation from *n*-hexane afforded pure (*Z*)-**7** in >99% de (by ^{1}H NMR). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.25 (s, 1H), 8.15-8.07 (m, 1H), 7.03-6.96 (m, 1H), 3.92 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 163.1, 156.6 (ddd, J = 252.4, 9.2, 2.6 Hz), 151.2 (ddd, J = 257.2, 14.9, 12.8 Hz), 146.5 (ddd, J = 245.4, 12.6, 3.5 Hz), 131.3 (m), 118.5 (ddd, J = 13.8, 6.6, 4.5 Hz), 117.6 (ddd, J = 21.2, 2.9, 1.6 Hz), 116.2 (m), 105.9 (dd, J = 28.0, 21.2 Hz), 53.9. ^{19}F NMR (470 MHz, $CDCl_3$): δ –113.6 (dd, J = 14.4, 5.8 Hz), –128.2 (dd, J = 21.8, 5.8 Hz), –141.0 (dd, J = 21.8, 14.4 Hz). GC-MS (EI): t_R = 18.96 min, *m/z* (%) = 296 (15, M^+), 294 (15, M^+), 265 (9), 263 (9), 215 (100), 183 (52), 156 (92).

(*Z*)-2-bromo-3-(2,4,5-trifluorophenyl)acrylic acid (18)
Bromoacrylate **7** (500 mg, 1.7 mmol) was dissolved in THF containing water (8 mL, 2.5% *v*/*v*). Et_3N (0.52 g, 5.1 mmol) was added, followed by the addition of LiBr (1.5 g, 17 mmol). The mixture was stirred vigorously at room temperature for 12 h, then poured into water, neutralized, and extracted with EtOAc (3 × 5 mL). The organic phase was dried with Na_2SO_4 and the solvent was removed under reduced pressure to give bromoacid **18**. White solid, yield 0.39 g (81%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.12 (s, 1H), 8.08-8.00 (m, 1H), 7.75-7.67 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 163.2, 155.4 (dd, J = 250.1, 10.8 Hz), 150.0 (dt, J = 253.1, 15.3 Hz), 145.7 (ddd, J = 242.6, 12.5, 3.3 Hz), 131.0, 119.2, 118.8 (m), 117.4 (d, J = 20.9 Hz), 106.5 (dd, J = 28.4, 21.7 Hz). ^{19}F NMR (470 MHz, DMSO-d_6): δ –113.2 (dd, J = 14.8, 6.1 Hz), –130.5 (dd, J = 23.0, 6.1 Hz), –142.0 (dd, J = 23.0, 14.8 Hz).

Biocatalytic procedures to D-2

Reductive amination of 5 with DAADH/GDH
Ketoacid **5** (109 mg, 0.5 mmol), NH_4Cl (134 mg, 2.5 mmol, 5.0 equiv.) and D-glucose (360 mg, 2.0 mmol, 4.0 equiv.) were dissolved in Na_2CO_3 buffer (10 mL, 100 mM, pH 9.0) containing $NADP^+$ (1 mM). *E. coli* cells co-producing DAADH and GDH (final conc. 100 mg mL^{-1}) were added, and the mixture was incubated at 37°C for 12 h with shaking (180 rpm). Complete conversion was confirmed by HPLC. Purification by ion exchange as described in the general section below afforded 89 mg (81% yield) D-**2** as a white solid.

Transamination of 5 with DAAT
Ketoacid **5** (109 mg, 0.5 mmol) and D-aspartic acid (100 mg, 0.75 mmol, 1.5 equiv.) were dissolved in KP_i buffer (20 mL, 100 mM, pH 8.0) containing PLP (1 mM). *E. coli* cells producing DAAT-T242G (final conc. 50 mg mL^{-1}) were added, and the mixture was incubated at 37°C for 12 h with shaking (180 rpm). Complete conversion was confirmed by HPLC. The suspension was heat-treated to inactivate the enzymes (95°C, 5 min) then centrifuged to remove insoluble materials (4000 rpm, 10 min). In order to remove unreacted D-aspartic acid, *E. coli* producing DDO (final conc. 50 mg mL^{-1}) were added to the supernatant, and the mixture was incubated at 37°C for 8 h with shaking (180 rpm). Purification by ion exchange as described in the general section below afforded 82 mg (75% yield) D-**2** as a white solid.

Deracemisation of DL-2 with LAAD and DAADH/GDH
Amino acid DL-**2** (110 mg, 0.5 mmol), NH_4Cl (134 mg, 2.5 mmol, 5.0 equiv.) and D-glucose (360 mg, 2.0 mmol, 4.0 equiv.) were dissolved in Na_2CO_3 buffer (10 mL, 100 mM, pH 9.0) containing $NADP^+$ (1 mM). *E. coli* cells producing LAAD (final conc. 50 mg mL^{-1}) and *E. coli* cells co-producing DAADH and GDH (final conc. 50 mg mL^{-1}) were added, and the mixture was incubated at 37°C for 12 h with shaking (180 rpm). Complete deracemisation was confirmed by HPLC. Purification by ion exchange as described in the general section below afforded 86 mg (79% yield) D-**2** as a white solid.

Deracemisation of DL-2 with LAAD and DAAT
Amino acid DL-**2** (110 mg, 0.5 mmol) and D-aspartic acid (67 mg, 0.5 mmol, 2.0 equiv. with respect to L-**2**) were dissolved in KP_i buffer (5 mL, 100 mM, pH 8.0) containing PLP (1 mM). *E. coli* cells producing LAAD (final conc. 50 mg mL^{-1}) and *E. coli* cells producing DAAT-T242G (final conc. 50 mg mL^{-1}) were added, and the mixture was incubated at 37°C for 12 h with shaking (180 rpm). Complete deracemisation was confirmed by HPLC. The suspension was heat-treated to inactivate the enzymes (95°C, 5 min) then centrifuged to remove insoluble materials (4000 rpm, 10 min). In order to remove unreacted D-aspartic acid, *E. coli* producing DDO (final conc. 50 mg mL^{-1}) were added to the supernatant, and the mixture was incubated at 37°C for 8 h with shaking (180 rpm). Purification by ion exchange as described in the general section below afforded 80 mg (74% yield) D-**2** as a white solid.

Hydroamination and stereoinversion of 6 with PAL, LAAD and DAAT
Cinnamic acid **6** (101 mg, 0.5 mmol) was dissolved in aqueous NH_3 solution (10 mL, 2.5 M, pH 8.0 adjusted by addition of dry ice). *E. coli* cells producing AvPAL (final conc. 50 mg mL^{-1}) were added and the mixture was incubated at 37°C for 12 h with shaking (180 rpm). The suspension was diluted with water (10 mL), then D-aspartic acid (133 mg, 1.0 mmol, 2.0 equiv.), *E. coli* cells producing LAAD (final conc. 50 mg mL^{-1}) and *E. coli* cells producing DAAT-T242G (final conc. 50 mg mL^{-1}) were added. The mixture was incubated at 37°C for 8 h with shaking (180 rpm). Complete deracemisation was confirmed by HPLC. The suspension was heat-treated to inactivate the enzymes (95°C, 5 min) then centrifuged to remove insoluble materials (4000 rpm, 10 min). In order to remove unreacted D-aspartic acid, *E. coli* producing DDO (final conc. 50 mg mL^{-1}) were added to the supernatant, and the mixture was incubated at 37°C for 8 h with shaking (180 rpm). Purification by ion exchange as described in the general section below afforded 73 mg (67% yield) D-**2** as a white solid.

Reduction of (*Z*)-7 to (*S*)-15 with ERED and GDH
Crude cell lysate containing ERED (6.0 mL), crude cell lysate containing GDH (2.0 mL), D-glucose (244 mg, 1.36 mmol, 4.0 equiv.) and $NADP^+$ (8 mg, final conc. 0.5 mM) were added to KP_i buffer (final volume 20 mL, 50 mM, pH 7.0). A solution of bromoester (*Z*)-**7** (110 mg, 0.34 mmol) in DMSO (800 μL) was added, and the mixture was incubated at 30°C for 5 d with shaking (150 rpm). The reaction was followed by GC-MS and or NMR until complete conversion was reached (by GC-MS). The product was extracted with EtOAc (3 × 10 mL), the organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure to give a residue, which was used without further purification. Yellow solid, yield 93 mg (84%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.12-7.04 (m, 1H), 6.96-6.88 (m, 1H), 4.43 (t, J = 7.6 Hz, 1H), 3.77 (s, 3H), 3.41 (dd, J = 14.4, 7.7 Hz, 1H), 3.25 (dd, J = 14.4, 7.7 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 169.5, 156.3 (ddd, J = 245.4, 9.4, 2.8 Hz), 149.6 (ddd, J = 251.6, 13.9, 10.5 Hz), 146.7 (ddd, J = 245.3, 12.7, 3.8 Hz), 120.2 (ddd, J = 17.7, 5.6, 4.2 Hz), 119.5 (ddd, J = 19.5, 5.8, 1.3 Hz), 105.8 (dd, J = 28.0, 20.9 Hz), 53.2, 42.9, 34.2. GC-MS (EI): t_R = 16.04 min, *m/z* (%) = 239 (7), 237 (7), 217 (100), 185 (62), 158 (31), 145 (50).

Conversion of (*S*)-15 to D-2

NaN_3 (14.8 mg, 0.23 mmol) was added portionwise to a solution of (*S*)-**15** (45 mg, 0.15 mmol) in DMF (2 mL), and the mixture was stirred at room temperature for 3 h. The reaction mixture was poured into water (4 mL) and extracted with EtOAc (3 × 1 mL). The organic phase was dried with Na_2SO_4, and the solvent was removed under reduced pressure to give methyl (*R*)-2-azido-3-(2,4,5-trifluorophenyl)propanoate ((*R*)-**16**) as a solid, which was used without further purification. Yellowish solid, yield 36 mg (93%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.13-7.03 (m, 1H), 6.98-6.88 (m, 1H), 4.12 (dd, J = 8.6, 5.6 Hz, 1H), 3.81 (s, 3H), 3.16 (dd, J = 14.4, 5.6 Hz, 1H), 2.96 (dd, J = 14.4, 8.6 Hz, 1H). GC-MS (EI): t_R = 16.75 min, *m/z* (%) = 231 (7), 216 (15), 171 (13), 145 (100). A solution of (*R*)-**16** (36 mg, 0.14 mmol) in EtOAc (3 mL) and Pd/C catalyst 10% *w/w* (1 mg) was stirred under H_2 atmosphere until complete reduction (by GC-MS). The solution was filtered and concentrated under reduced pressure to give methyl (*R*)-2-amino-3-(2,4,5-trifluorophenyl)propanoate ((*R*)-**17**) as a solid, which was used without further purification. Yellowish solid, yield 30.9 mg (95%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.10-7.02 (m, 1H), 6.94-6.87 (m, 1H), 3.72 (s, 3H), 3.71 (dd, J = 7.8, 5.5 Hz, 1H) 3.03 (dd, J = 13.9, 5.5 Hz, 1H), 2.84 (dd, J = 13.9, 7.8 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 175.1, 156.3 (ddd, J = 244.4, 9.5, 2.9 Hz), 149.1 (ddd, J = 250.5, 14.4, 12.4 Hz), 146.6 (ddd, J = 236.1, 12.9, 3.8 Hz), 120.9 (ddd, J = 18.2, 5.4, 4.4 Hz), 119.2 (ddd, J = 19.0, 6.1, 1.1 Hz), 105.6 (dd, J = 28.6, 20.7 Hz), 54.7, 52.3, 34.0. GC-MS (EI): t_R = 15.72 min, *m/z* (%) = 174 (95), 145 (29), 127 (32), 88 (100). Crude (*R*)-**17** (30.9 mg) was refluxed in conc. HCl (2 mL, 37% *w/v*) for 4 h, affording complete hydrolysis to derivative D-**2**. Purification by ion exchange as described in the general section below afforded 18.3 mg (56% yield from (*S*)-**15**) D-**2** as a yellowish solid.

Purification of D-2 from aqueous reaction mixtures

The crude reaction mixture containing D-**2** was acidified to pH < 2.0 by addition of aqueous H_2SO_4 (10% *w/v*) and centrifuged (4000 rpm, 10 min). Dowex 50WX8 hydrogen form (2.0 g) was washed with deionized water (30 mL) and aqueous H_2SO_4 (20 mL, 10% *w/v*). The acidified supernatant from the biotransformation was loaded on to the resin (1 mL min^{-1}). The resin was washed with deionized water (until pH ~7.0), then the product was eluted with aqueous NH_4OH (30 mL, 10% *w/v*). Fractions containing the product were pooled and evaporated in a centrifugal evaporator, to afford amino acid D-**2** as a white or pale yellow solid.

REFERENCES

1 International Diabetes Federation (IDF), *Diabetes Atlas*, 8th ed., 2017, http://www.diabetesatlas.org (last accessed 16/05/2018).

2 D. Kim, L. Wang, M. Beconi, G. J. Eiermann, M. H. Fisher, H. He, G. J. Hickey, J. E. Kowalchick, B. Leiting, K. Lyons, F. Marsilio, M. E. McCann, R. A. Patel, A. Petrov, G. Scapin, S. B. Patel, R. S. Roy, J. K. Wu, M J. Wyvratt, B. B. Zhang, L. Zhu, N. A. Thornberry, A. E. Weber, *J. Med. Chem.*, 2005, **48**, 141-151.

3 A. A. Desai, *Angew. Chem. Int. Ed.*, 2011, **50**, 1974-1976.

4 C. K. Savile, J. M. Janey, E. C. Mundorff, J. C. Moore, S. Tam, W. R. Jarvis, J. C. Colbeck, A. Krebber, F. J. Fleitz, J. Brands, P. N. Devine, G. W. Huisman, G. J. Hughes, *Science*, 2010, **329**, 305-309.

5 (a) S. G. Davies, A. M. Fletcher, J. E. Thomson, *Tetrahedron: Asymmetry*, 2015, **26**, 1109-1116; (b) C. M. Alcántara, A. R. Alcántara, *Biocatal. Biotransform.*, 2018, **36**, 12-46.

6 C. S. Subbaiah, W. Haq, *Tetrahedron: Asymmetry*, 2014, **25**, 1026-1030.

7 N. J. Turner, E. O'Reilly, *Nat. Chem. Biol.*, 2013, **9**, 285-288.

8 K. Vedha-Peters, M. Gunawardana, J. D. Rozzell, S. J Novick, *J. Am. Chem. Soc.*, 2006, **128**, 10923-10929.

9 C. J. W. Walton, F. Parmeggiani, J. E. Barber, J. L. McCann, N. J. Turner, R. A. Chica, *ChemCatChem*, 2018, **10**, 470-474.

10 F. Parmeggiani, S. T. Ahmed, M. T. Thompson, N. J. Weise, J. L. Galman, D. Gahloth, M. S. Dunstan, D. Leys, N. J. Turner, *Adv. Synth. Catal.*, 2016, **358**, 3298-3306.

11 (a) D. Tessaro, G. Molla, L. Pollegioni, S. Servi, Chemo-enzymatic deracemization methods, in: Modern biocatalysis: stereoselective and environmentally friendly reactions, W. D. Fessner, T. Anthonsen, Eds., 2009, Wiley-VCH, Weinheim; (b) C. C. Gruber, I. Lavandera, K. Faber, W. Kroutil, *Adv. Synth. Catal.* 2006, **348**, 1789-1805.

12 G. Molla, R. Melis, L. Pollegioni, *Biotechnol. Adv.*, 2017, **35**, 657-668.

13 F.-R. Alexandre, D. P. Pantaleone, P. P. Taylor, I. G. Fotheringham, D. J. Ager, N. J. Turner, Tetrahedron Lett., 2002, 43, 707-710.

14 A. Matsushima, T. Fujita, K. Okada, N. Shirasu, T. Nose, Y. Shimohigashi, *Bull. Chem. Soc. Jpn.*, 2000, **73**, 2531-2538.

15 (a) F. Parmeggiani, N. J. Weise, S. T. Ahmed, N. J. Turner, *Chem. Rev.*, 2018, **118**, 73-118; (b) M. M. Heberling, B. Wu, S. Bartsch, D. B. Janssen, *Curr. Opin. Chem. Biol.*, 2013, **17**, 250-260.

16 S. L. Lovelock, R. C. Lloyd, N. J. Turner, *Angew. Chem. Int. Ed.*, 2014, **53**, 4652-4656.

17 F. Parmeggiani, S. L. Lovelock, N. J. Weise, S. T. Ahmed, N. J. Turner, *Angew. Chem., Int. Ed.*, 2015, **54**, 4608-4611.

18 M. C. Moffitt, G. V. Louie, M. E. Bowman, J. Pence, J. P. Noel, B. S. Moore, *Biochemistry*, 2007, **46**, 1004-1012.

19 D. S. Hodgins, *J. Biol. Chem.*, 1971, **246**, 2977-2985.

20 (a) N. J. Weise, S. T. Ahmed, F. Parmeggiani, J. L. Galman, M. S. Dunstan, S. J. Charnock, D. Leys, N. J. Turner, *Sci. Rep.*, 2017, **7**, 13691; (b) S. T. Ahmed, F. Parmeggiani, N. J. Weise, S. L. Flitsch, N. J. Turner, *ACS Catal.*, 2018, **8**, 3129-3132.

21 (a) H. S. Toogood, N. S. Scrutton, *ACS Catal.*, 2018, **8**, 3532-3549, (b) H. S. Toogood, N. S. Scrutton, *Curr. Opin. Chem. Biol.*, 2014, **19**, 107-115.

22 (a) E. Brenna, F. G. Gatti, A. Manfredi, D. Monti, F. Parmeggiani, *Eur. J. Org. Chem.*, 2011, 4015-4022; (b) E. Brenna, F. G. Gatti, A. Manfredi, D. Monti, F. Parmeggiani, *Org. Process Res. Dev.*, 2012, **16**, 262-268; (c) G. Tasnádi, C. K. Winkler, D. Clay, N. Sultana, W. M. F. Fabian, M. Hall, K. Ditrich, K. Faber, *Chem. Eur. J.*, 2012, **18**, 10362-10367.

23 M. M. Kayser, J. Zhu, D. L. Hooper, *Can. J. Chem.*, 1997, **75**, 1315-1321.

24 (a) R. A. Sheldon, *Chem. Ind.* 1992, 903-907; (b) R. A. Sheldon, *Green Chem.*, 2017, **19**, 18-43.

25 F. Roschangar, R. A. Sheldon, C. H. Senayanake, *Green Chem.*, 2015, **17**, 752-768.