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ACS Catal., **Just Accepted Manuscript** • DOI: 10.1021/acscatal.6b00686 • Publication Date (Web): 05 May 2016

Biocatalytic Transamination for the Asymmetric Synthesis of Pyridylalkylamines. Structural and Activity Features in the Reactivity of Transaminases

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ABSTRACT. A set of transaminases has been investigated for the biocatalytic amination of 1-(4-chloropyridin-2-yl)alkan-1-ones. The influence of the chain length of the *n*-1-alkanone at the C-2 position of the pyridine has been studied in the reaction with different (*R*)- and (*S*)-selective transaminases. Thus, enantiopure amines were isolated with high purity starting from a wide selection of prochiral ketones. On the one hand excellent yields (97->99% conversion, up to 93% isolated yield) and stereoselectivity values (>99% *ee* for both amine enantiomers) were found for *n*-1-alkanone linear short chain substituents such as ethanone or propanone. On the other hand, more hindered substrates were accepted only when using evolved enzymes such as an evolved variant of (*R*)-*Arthrobacter* (ArRmut11-TA). An initial common structural feature was the presence of a chlorine atom on the C-4 position of the pyridine core, which was found to increase the reactivity of the starting ketone, giving extra versatility for the introduction of other chemical functionalities towards more complex and applicable organic molecules. In order to gain a deeper understanding about the substrate specificity of different transaminases, additional structural features were considered by variation of the acetyl group position on the pyridine ring and the use of related acetophenone derivatives.

KEYWORDS. Transaminases; amines; biocatalysis; enzymes; substrate specificity; transamination

INTRODUCTION

The asymmetric synthesis of amines attracts significant attention due to their multiple immediate applications and versatility as building blocks in the preparation of bioactive molecules.[1] In

addition, amines have been widely employed as chiral auxiliaries, asymmetric catalysts and chiral resolving agents.[2]

Enzymes offer highly sustainable and efficient synthetic alternatives to current existing chemical methods,[3] providing access to enantiopure amines and derivatives with excellent selectivity.[4] Traditionally, hydrolases have been the biocatalysts of choice for the preparation of optically active amines through classical or dynamic kinetic resolutions of the corresponding racemates,[5] but the development of other classes of enzymes such as amine oxidases,[6] transaminases,[7] berberine bridge enzymes,[8] amine dehydrogenases[9] and more recently imine reductases[10] presents biotransformations as versatile tools for the development of highly efficient asymmetric transformations to prepare these compounds.

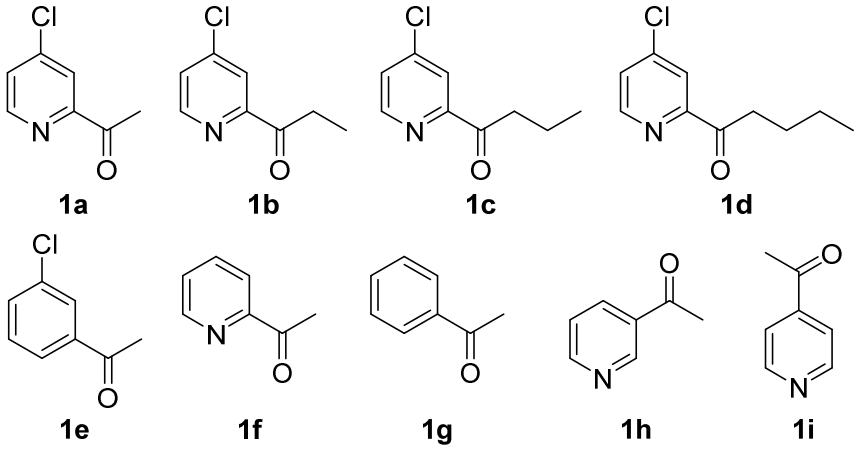
Transaminases (TAs), also known as aminotransferases (EC 2.6.1.X) or amine transaminases, are pyridoxal 5'-phosphate (PLP) dependent enzymes[11] that catalyze the kinetic resolution of racemic amines and, conversely, asymmetric synthesis of amines from the corresponding prochiral ketones.[7,12] The latter reaction is preferred over the first as it culminates in a 100% theoretical maximum yield. In this sense and taking into account thermodynamic considerations, the main limitation of an unfavorable equilibrium shift towards the formation of the amine has been broadly studied and overcome by diverse strategies.[13]

The pyridine core constitutes an interesting nitrogen-based scaffold, both from a biological and synthetic point of view.[14] It can participate in chemical transformations either *via* the nitrogen or *via* the aromatic ring, which makes pyridines attractive compounds for many different applications.[15] Particularly, the presence of a chlorine atom on the aromatic ring opens up a variety of synthetic possibilities for increasing molecular complexity, allowing for instance the

preparation of enantiopure 4-(*N*,*N*-dimethylamino)-2-(1-hydroxyalkyl)pyridines using lipases[16] or alcohol dehydrogenases,[17] compounds that offer interesting applications in asymmetric organocatalysis as ligands or chiral catalysts.[17]

Herein, we describe the enzymatic transamination of 1-(4-chloropyridin-2-yl)alkan-1-ones bearing different size alkyl chains on the C-2 position of the aromatic ring (**1a-d**, Figure 1 top). A range of transaminases overexpressed in *E. coli* was investigated to establish the optimal reaction conditions and study the influence of the substitution pattern on the reactivity of the prochiral ketones. Furthermore, additional structural parameters such as the type of aromatic ring, the presence of chlorine atoms or the position of the ketone functionality on the aromatic ring will be considered (structures **1e-i**, Figure 1 bottom), in order to provide an insight in the interaction between selected transaminases and aromatic substrates with different structural cores. Correlations between conversion of the enzymatic transamination processes and the infrared carbonyl stretch of the starting ketones will be done, discussing the best structural motifs to allow the displacement of the equilibrium towards the amine formation.

Figure 1. Chemical structure of 1-(4-chloropyridin-2-yl)alkan-1-ones (top) and structural modifications considered for the study of transaminase reactivity (bottom).

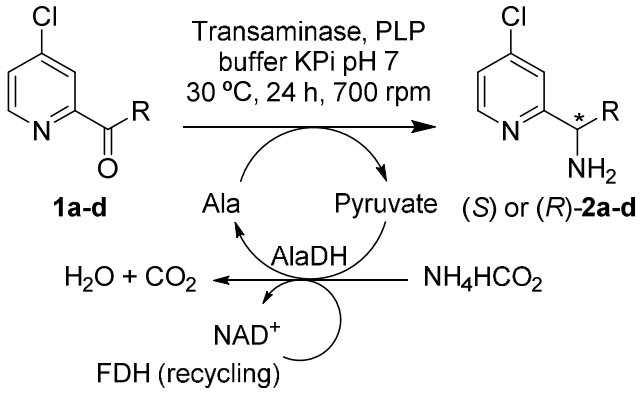


RESULTS AND DISCUSSION

Prochiral ketones **1a-d** were chemically synthesized starting from commercially available 4-chloropyridine *N*-oxide in a two-step sequence previously developed in our research group.[16a] The biocatalytic transamination of ketones **1a-d** was initially performed at a 50 mM substrate concentration using a set of TAs overexpressed in *E. coli* (Table 1): the (*S*)-selective TAs from *Vibrio fluvialis* (Vf)[18] and *Chromobacterium violaceum* (Cv),[19] or the (*R*)-selective TAs from (*R*)-*Arthrobacter* species (ArR)[20] and *Aspergillus terreus* (At).[21] L- or D-alanine (L/D-Ala), respectively, were initially used as amine source depending on the enzyme's selectivity, and the equilibrium was shifted towards amine formation by removing the pyruvate to L-alanine with alanine dehydrogenase (AlaDH) at the expense of ammonia and formate generating only CO_2 and H_2O as byproducts.[22]

As shown in Table 1, quantitative conversions and excellent stereoselectivities were observed for the methyl ketone **1a** with all tested TAs (entries 1-4). Similarly, access to both enantiomers of amine **2b** was accomplished by selecting the appropriate TA (entries 5-8). Thus, the enantiopure amine (*S*)-**2b** was obtained with quantitative conversion (entries 5 and 6) and the complementary (*R*)-antipode with very high conversions (up to 97%, entries 7 and 8).

Table 1. Enzyme-catalyzed asymmetric transamination of ketones **1a-d**.[a]



Entry	R	TA	*c* (%)[b]	*ee* (%)[c]
1	Me (**1a**)	Vf	>99	>99 (*S*)
2	Me (**1a**)	Cv	>99	>99 (*S*)
3	Me (**1a**)	ArR	>99	>99 (*R*)
4	Me (**1a**)	At	>99	>99 (*R*)
5	Et (**1b**)	Vf	>99	>99 (*S*)
6	Et (**1b**)	Cv	>99	>99 (*S*)
7	Et (**1b**)	ArR	95	>99 (*R*)
8	Et (**1b**)	At	97	>99 (*R*)
9	^{n}Pr (**1c**)	Vf	72	>99 (*S*)
10	^{n}Pr (**1c**)	Cv	14	>99 (*S*)
11	^{n}Pr (**1c**)	ArR	3	n.m.
12	^{n}Pr (**1c**)	At	<1	n.m.
13	^{n}Bu (**1d**)	Vf	15	81 (*S*)
14	^{n}Bu (**1d**)	Cv	19	<1
15	^{n}Bu (**1d**)	ArR	<1	n.m.
16	^{n}Bu (**1d**)	At	<1	n.m.

[a] Reaction conditions: phosphate buffer (100 mM, pH 7), ketone **1a-d** (50 mM), lyophilized cells *E. coli*/TA (20 mg), PLP (1 mM), NAD^+ (1 mM), L- or D-alanine (250 mM), AlaDH (10 μL, 11

U), formate dehydrogenase (FDH, 2.6 mg, 11 U), ammonium formate (150 mM), 24 h at 30 ºC and 700 rpm.

[b] Conversion values were determined by GC analysis after isolation of products by extraction from basified media (See experimental section).

[c] Enantiomeric excess values were measured by GC or HPLC (See Supporting Information) on a chiral phase. n.m.: not measured. The stereochemistry of the products, which appear in brackets, was confirmed by comparing the corresponding optical rotations with the values reported in literature (See experimental section).

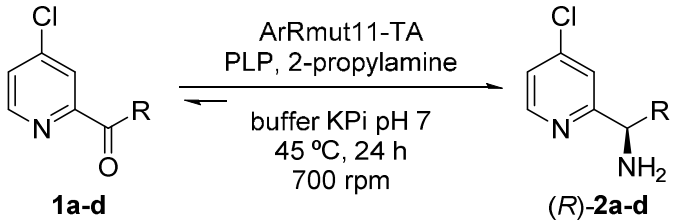
The exquisite preliminary results encouraged us to carry out representative examples on preparative scale (50 mg of substrate). Consequently, transamination reactions of methyl ketone **1a** with Cv-TA, allowed the isolation of (*S*)-**2a** (*ee* >99%) in 82% isolated yield. The synthetic potential of the Vf-TA was also demonstrated obtaining the same enantiopure amine in 93% isolated yield (see Experimental Section). Similarly, Vf-TA led to quantitative transformation and 93% isolated yield of (*S*)-**2b** after a simple extraction protocol. The isolated amines were analyzed by GC and NMR techniques, **2a** and **2b** being obtained with very high purity without no additional purification.

A dramatic decrease of conversion was observed when bulkier ketones were considered containing longer alkyl rests (**1c** and **1d**, propyl and butyl, respectively), which is in agreement with previous TA-catalyzed biotransamination examples with structurally similar acetophenone derivatives.[23] Nevertheless, amine (*S*)-**2c** was still obtained with good conversion and in enantiopure form when Vf- TA was used (entry 9), while low conversions were attained for the (*S*)-selective Cv-TA or the (*R*)-selective enzymes (entries 10-12). Other TAs (*Arthrobacter citreus*, *Bacillus megaterium*, *Paracoccus denitrificans*, *Pseudomonas fluorescens* or

Hyphomonas neptunium) proved to be not suitable. Low or no detectable conversion was obtained for the pentanone **1d** in all cases (entries 13-16).

Since none of the enzymes tested provided an efficient access to the more hindered amines, an evolved variant of (*R*)-*Arthrobacter* (ArRmut11-TA) was explored, also displaying (*R*)-selectivity as the wild-type enzyme. This enzyme has been previously successfully applied in the transamination of sterically demanding substrates,[24] and presents additional advantages as its high thermal stability and tolerance to 2-propylamine as amine source. Thus, the reaction was run at elevated temperature (45 ºC) circumventing a recycling system by adding an excess of the amine donor. The results are summarized in Table 2.

Table 2. Asymmetric transamination of ketones **1a-d** using the ArRmut11-TA.[a]



1a-d (*R*)-**2a-d**

Entry	R	TA preparation	*c* (%)[b]	*ee* (%)[c]
1	^{n}Bu (**1d**)	crude	64	>99
2	^{n}Bu (**1d**)	semipurified	87	>99
3	^{n}Pr (**1c**)	crude	66	>99
4	^{n}Pr (**1c**)	semipurified	96	>99
5	Et (**1b**)	semipurified	97	>99
6	Me (**1a**)	semipurified	98	99

[a] Reaction conditions: phosphate buffer (100 mM, pH 7), ketone **1a-d** (50 mM), ArRmut11-TA (20 mg of crude enzyme or 500 µL of semipurified version), PLP (0.5 mM), 2-propylamine (1 M), 24 h at 45 ºC and 700 rpm.

[b] Conversion values were determined by GC analysis after isolation of products by extraction from basified media (See experimental section).

[c] Enantiomeric excess values were measured by GC or HPLC (See Supporting Information) on a chiral phase.

Good conversions and excellent enantiomeric excesses were achieved with the ArRmut11-TA crude preparation for the formation of (*R*)-amines **2d** and **2c** (entries 1 and 3). Next, a semipurified preparation of the enzyme was tested aiming for an improvement of the conversion values. Indeed, very high conversions were detected (84-98%) without affecting the selectivity of the reaction. As a proof of concept, scale up of the process was performed using 50 mg of substrate **1c**, recovering the enantiopure amine (*R*)-**2c** in 68% isolated yield after an extraction step. The biocatalytic system was also successfully used for the preparation of amines bearing short alkyl chains (**2a-b**, R=Me, Et) reaching completion within 24 h (entries 6 and 5, respectively).

In order to get a deeper insight about the structure-activity relationship for TAs, additional enzymatic studies were performed by considering structural parameters such as: (i) chain length of the alkyl group, (ii) presence of nitrogen and chlorine atoms in/on the aromatic ring; (iii) the position of substitution of the acyl group on the pyridine ring.

The transamination of ketones **1a-d** was followed over time catalyzed by the (*S*)-selective Vf-TA (Figure 2), which displayed a good activity even for the butanone **1c** (72% conversion, entry 9 in Table 1). The ethanone **1a** (R=Me) was converted very quickly under the reaction conditions employed reaching 74% conversion in 1 hour, and almost complete consumption of the ketone after 7 hours. On the other hand, propanone **1b** (R=Et) was transformed more slowly. Reaction

progress was similar for substrates **1b** and **1c** at 1 and 3 hours (5 vs. 3% and 14 vs. 12% conversion, respectively), the difference between these two ketones became more significant after 5 hours (34 vs. 18% conversion) and 7 hours (44 vs. 26% conversion). In line with the conversions achieved before (entry 13 in Table 1), the pentanone **1d** (R=Bu) only reached 4% of conversion at 7 hours, which clearly illustrates the decrease of reactivity for substrates bearing medium-large aliphatic chains.

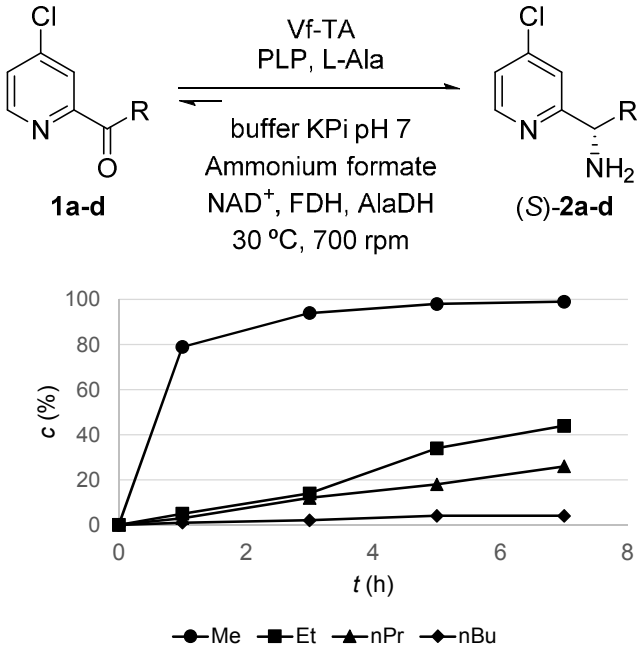
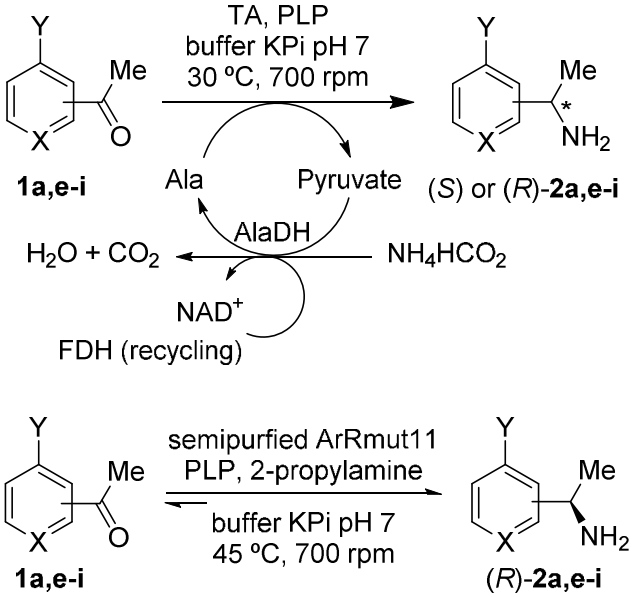


Figure 2. Graphical representation of Vf-TA reactivity as function of the chain size of ketones **1a-d** after 1, 3, 5 and 7 hours.

Following our aim to establish a structure-activity relationship for TAs, we focused next on other structural aspects related to the aromatic ring, including the presence of heteroatoms (nitrogen and chlorine) and the position of the acetyl group on the pyridine ring. Therefore, ethanone **1a** was chosen as model substrate and methyl ketones **1e-i** were included in this study (Figure 1).

Amination experiments were carried out employing transaminases of complementary stereopreference, under previously optimized reaction conditions (temperature, substrate concentrations, amine donors, recycling systems, reaction time, pH and type of buffer) for an accurate comparison between substrates (Scheme 1).



Scheme 1. Enzymatic transamination of **1a** and **1e-i** using alanine or 2-propylamine as amino donor.

The amination of **1a**,**e-i** was performed using various TAs (10 mg *E. coli*/TA, 1 h) at 30 ºC for Vf, Cv, ArR, while 45 ºC was selected for the more thermostable ArRmut11-TA used as semipurified preparation (500 µL, 8 U, Figure 3). From the results obtained some general facts concerning their reactivity can be concluded. A decrease in conversion values was detected when comparing the model substrate 1-(4-chloropyridin-2-yl)ethan-1-one (**1a**, conversion up to 87%) with 2-acetylpyridine (**1f**, conversion up to 50%), which lacks the chlorine atom on the C-4 position. This drop in the conversion became more significant when investigating aromatic

derivatives such as 3'-chloroacetophenone (**1e**, conversion up to 17%) and acetophenone (**1g**), which reached the highest conversion (21%) using the (*R*)-selective ArR-TA. A similar tendency was observed with 3-acetylpyridine (**1h**, conversion up to 19%), while no reaction was found with semipurified ArRmut11-TA. Finally, the amination of 4-acetylpyridine (**1i**) was studied, finding a similar trend with respect to 2-acetylpyridine (**1f**), reaching the best conversion (42% and 57%) with the Cv- and ArR-TAs, respectively. In any case, there are major differences compared to 1-(4-chloropyridin-2-yl)ethan-1-one (**1a**) and in fact conversions for 4-acetylpyridine (**1i**) are just comparable to those for 2-acetylpyridine (**1f**).

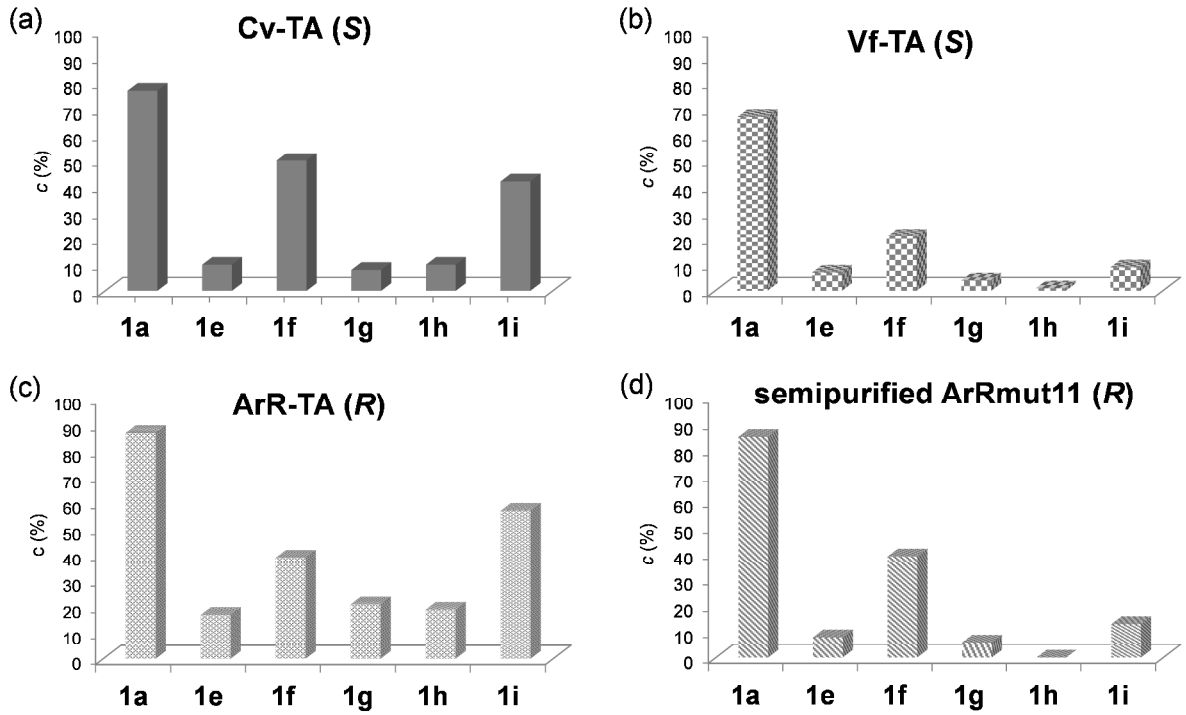


Figure 3. Reactivity comparison for the amination of the methyl ketones **1a,e-i** with (a) Cv, (b) Vf and (c) ArR-TAs (30 ºC, 1 h), and (d) ArRmut11-TA (45 ºC, 1 h).

Interestingly, an analysis of the spectroscopical data revealed a positive correlation between conversions and vibrational frequencies of the carbonyl band in the infrared spectra for ketones

1a,**e-i**, and therefore with the C=O bond length.[25] The conversions achieved with Cv-TA for various substrates presented in Figure 4 shows this correlation with the electronic effect of the chlorine atom and the pyridine nitrogen, the last being the predominant one. The pyridine derivatives **1a**, **1f** and **1i** bearing the acetyl group at C-2 or C-4 positions, in which the electron withdrawing effect of the nitrogen is stronger, displayed frequencies higher than 1690 cm^{-1}, those compounds leading to higher conversion values. Among them, the pyridine **1a** is the more reactive one as it has the additional electron withdrawing effect of the chlorine. On the contrary, the larger C=O bonds fit to the acetophenone derivatives **1g** and **1e**, and to the pyridine **1h**, in which the conversion of the reaction reached a maximum 10% after 1 hour. The acetophenone **1e** with a chlorine atom and the pyridine **1h** displayed a slight higher reactivity than acetophenone (**1g**), which does not present the electron withdrawing effects of **1e** and **1h**.

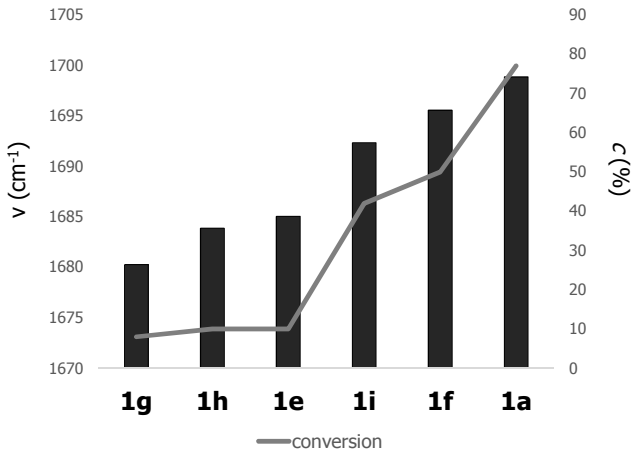
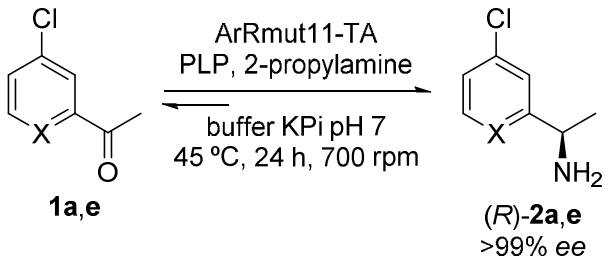


Figure 4. Graphical representation of vibrational frequencies of the carbonyl IR band (bars) towards conversion values (line) in the enzyme-catalyzed transamination of **1a** and **1e-i** using Cv-TA (700 rpm, 30 ºC and 1 hour).

Finally, the high conversions observed for **1a** within 1 hour using semipurified ArRmut11-TA (85% conversion, Figure 3d) might indicate a higher thermodynamic stability of the product amine **2a**, which may facilitate a shift of the equilibrium towards its formation. In this case, less amount of the amine donor (2-propylamine) would be required for a practical biotransformation. Accordingly, the concentration of 2-propylamine was reduced from 1 M to 100 mM (Table 3). In addition, the same experiment was made employing 3'-chloroacetophenone (**1e**).

Table 3. Asymmetric enzyme-catalyzed transamination of **1a** and **1e** using semipurified ArRmut11-TA.[a]



Entry	X	[2-propylamine] (M)	t (h)	*c* (%)[b]
1	N (**1a**)	1	1	85
2	N (**1a**)	1	24	98
3	N (**1a**)	0.1	24	76
4	CH (**1e**)	1	1	39
5	CH (**1e**)	1	24	53
6	CH (**1e**)	0.1	24	24

[a] Reaction conditions: phosphate buffer (100 mM, pH 7), ketone **1a** and **1e** (50 mM), ArRmut11-TA (500 µL), PLP (0.5 mM), 2-propylamine (0.1-1 M), 1-24 h at 45 ºC and 700 rpm.

[b] Conversion values were determined by GC analysis after isolation of products by extraction from basified media (See experimental section).

Differences between substrate **1a** and **1e** are for instance the higher conversion for **1a** (85% vs. 39%) after 1 hour at 1 M of 2-propylamine (entries 1 and 4), something already discussed. Furthermore, at 100 mM 2-propylamine concentration, ketone **1e** reached 24% conversion after 24 h (entry 6), while the reaction with **1a** achieved 76% (entry 3). The values obtained for **1e** are in agreement with the fact that the deamination reaction is favored for acetophenone derivatives compared to the reverse amination reaction.[26] On the other hand, the excellent results obtained for the amination of **1a** even with low excesses of amine donor are consistent with thermodynamic preference for the formation of amine (*R*)-**2a**. This inclination was analyzed through different perspectives. The first attempt to explain it focuses on an intramolecular hydrogen bond between the amino group and the pyridine nitrogen which would provide an additional stabilization to the amine and therefore drive the reaction to the amination direction.[27] Computational studies[28] on the model substrate **2a** confirmed the presence of the hydrogen bond (Figure S4, for further details see the Supporting Information).

Alternatively, energy calculations supported the thermodynamic stabilization by the difference observed in the ΔG of the reaction for the amination of both ketones **1a** and **1e**, the pyridine transformation being 4.3 kJ/mol more favorable (see Table S1 and Figure S3).

CONCLUSIONS

The asymmetric synthesis of 1-(4-chloropyridin-2-yl)alkan-1-amines has been performed through transaminase-catalyzed reactions starting from their corresponding prochiral ketones. Hence, ethan- and propanone derivatives were successfully transformed with a variety of (*R*)- and (*S*)-selective transaminases. After finding adequate reaction conditions, both amine antipodes have been obtained with excellent conversion values and high to excellent isolated

yields in preparative biotransformations, recovering the desired amines with high purity after a simple extraction protocol. To achieve full stereoselectivity and high conversion for the most hindered pyridine substrates, the use of an evolved variant from (*R*)-*Arthrobacter* species was required. The 1-(4-chloropyridine-2-yl)ethan-1-one **1a** was transformed very efficiently even at reduced concentration of amine donor most likely due to thermodynamic reasons as a result of the formation of an internal hydrogen bond. Different structural parameters including pyridine and acetophenone derivatives, some bearing chlorine atoms at the aromatic ring, and with the alkanone rest at variable positions in the aromatic ring were examined, which enabled to identify ideal structural motives for the substrate to be converted by the enzymes investigated.

EXPERIMENTAL SECTION

1-(4-Chloropyridine-2-yl)alkan-1-ones **1a-d** and racemic amines **2a-d** were prepared according to previously reported procedures from 4-chloropyridine *N*-oxide.[16] Chemical reagents and solvents were used as received from commercial sources.

TLC analyses were carried out with precoated aluminium sheets (TLC silica gel 60 F254, Merck) with detection by UV or staining with potassium permanganate or ninhydrin. Preparative chromatographic separations were performed by flash chromatography on Merck silica gel (230-400 mesh). Optical rotation was measured at 20 °C with a Perkin-Elmer 241 polarimeter at the sodium D-line. ^{1}H and ^{13}C NMR spectra were recorded at 20 °C with a Bruker 300 MHz unit; chemical shifts are given in ppm relative to the resonance of the solvent. Analytical data and retention times for the measurement of conversion and enantiomeric excess values are given in the Supporting Information section. The purity of the resulting optically active amines obtained through transaminase-catalyzed processes was determined by GC and NMR analyses.

Formate dehydrogenase was purchased from Evocatal GmbH (lyophilized powder 4.1 U/mg) and NAD^+ free acid were purchased from Codexis Inc. Lyophilized *E. coli* cells containing overexpressed transaminases were prepared as previously reported.[20a,29] Lyophilized cells applied as catalyst displayed the following activities (determined for the deamination of α-methylbenzylamine with pyruvate): ArR: 2.2 U/mg, ArRmut11: 0.3 U/mg, At: 0.5 U/mg, Vf: 1.3 U/mg, Cv: 2.1 U/mg, purified L-alanine dehydrogenase: 1.1 U/μL. Small scale reactions were shaken in 2 mL thermo shaker Eppendorf® Comfort. Preparative scale reactions were performed in an Infors Unitron shaker.

Heat purification protocol for ArRmut11

Lyophilized *E. coli* cells containing overexpressed enzyme (2 g) were suspended in a 100 mM phosphate buffer pH 7 (20 mL) and the suspension was sonicated with the following protocol: 1 s pulse; 4 s pause; 2.5 min; 40% amplitude. The mixture was centrifuged for 20 min at 45000 g and 4 ºC and the supernatant was treated for 30 min at 50 ºC. After this time the suspension was centrifuged again for 20 min at 45000 g and 4 ºC and the supernatant containing the active enzyme was stored at -18 ºC. Protein concentration: 26.7 mg/mL according to Bradford's assay. Activity (determined for the deamination of α-methylbenzylamine with pyruvate): 16 U/mL.

Typical procedure for asymmetric transamination of ketones 1a-d employing alanine dehydrogenase as regeneration system

To a suspension of ketones **1a-d** (0.05 mmol, 50 mM) in a 100 mM phosphate buffer pH 7 (440 μL) were successively added ammonium formate (100 μL of 1.5 M solution in a 100 mM phosphate buffer pH 7; final concentration 150 mM), alanine (250 μL of 1 M solution in phosphate buffer 100 mM pH 7; final concentration 250 mM), NAD^+ (100 μL of 10 mM solution

in a 100 mM phosphate buffer pH 7; final concentration 1 mM), PLP (100 μL of 10 mM solution in a 100 mM phosphate buffer pH 7; final concentration 1 mM), lyophilized cells of *E. coli* containing overexpressed transaminases (20 mg), formate dehydrogenase (FDH, 2.6 mg, 11 U) and alanine dehydrogenase (AlaDH, 10 μL, 11 U). D- or L- alanine were used as amine donor depending on the (*R*) or (*S*)-transaminase selectivity, respectively.

The resulting mixture was shaken at 30 °C and 700 rpm for 24 h. After this time the reaction was quenched by adding aqueous NaOH 4 M (400 μL), extracted with ethyl acetate (3 x 500 μL), and organic phases were combined and dried with Na_2SO_4. Reaction crude was analyzed through GC to determine conversion values and, after an *in situ* derivatization, enantiomeric excess.

Typical experimental procedure for asymmetric transamination of ketones 3a-d catalyzed by semipurified ArRmut11-TA

Ketones **1a-d** (0.05 mmol, 50 mM) were suspended in a 100 mM phosphate buffer pH 7 (500 μL) containing PLP (1 mM; final concentration 0.5 mM) and isopropylamine (2 M; final concentration 1 M). Then, semipurified ArRmut11 (500 μL) was added and the mixture was shaken at 45 °C and 700 rpm for 24 h. The reaction was quenched by adding aqueous NaOH 4 M (400 μL), extracted with ethyl acetate (3 x 500 μL), and organic phases were combined and dried with Na_2SO_4. Reaction crude was analyzed through GC to determine conversion values and, after an *in situ* derivatization, enantiomeric excess.

General procedure for preparative reductive amination of 1a and 1b employing alanine dehydrogenase as regeneration system

To a suspension of ketones **1a** or **1b** (0.32 mmol, 50 mM) in a 100 mM phosphate buffer pH 7 (2.8 mL) were successively added ammonium formate (643 μL of 1.5 M solution in a 100 mM phosphate buffer pH 7; final concentration 150 mM), L-alanine (1.6 mL of 1 M solution in a 100 mM phosphate buffer pH 7; final concentration 250 mM), NAD^+ (643 μL of 10 mM solution in a 100 mM phosphate buffer pH 7; final concentration 1 mM), PLP (643 μL of 10 mM solution in a 100 mM phosphate buffer pH 7; final concentration 1 mM), lyophilized cells of *E. coli* containing overexpressed transaminases (32 mg for **1a**, 96 mg for **1b**), formate dehydrogenase (16.4 mg, 69 U) and AlaDH (63 μL, 69 U). The resulting mixture was shaken at 30 °C and 120 rpm for 24 h. The reaction was quenched by adding aqueous NaOH 4 M until pH ≈ 10 and extracted with ethyl acetate (3 x 10 mL). The organic phases were combined, dried with Na_2SO_4 and the solvent was removed under reduced pressure. Enantiopure amines (*S*)-**2a** and (*S*)-**2b** were isolated as colorless liquids in 82-93% yield. For (*S*)-**2a** in >99% *ee*: $[\alpha]_D^{20}$ = -23.4 (c 0.5, $CHCl_3$); lit. $[\alpha]_D^{20}$ = -20.4 (c 0.5, $CHCl_3$).[16b] For (*S*)-**2b** in >99% *ee*: $[\alpha]_D^{20}$ = -5.8 (c 0.6, $CHCl_3$); lit. $[\alpha]_D^{20}$ = -5.1 (c 0.6, $CHCl_3$).[16b]

Preparative reductive amination of 1c catalyzed by semipurified ArRmut11-TA

In a Falcon tube, ketone **1c** (50 mg, 0.27 mmol, 50 mM) was suspended in a 100 mM phosphate buffer pH 7 (2.7 mL) containing PLP (1 mM; final concentration 0.5 mM) and isopropylamine (2 M; final concentration 1 M). Then, semipurified ArRmut11-TA (2.7 mL) was added and the mixture was shaken at 45 °C and 120 rpm for 24 h. The reaction was quenched by adding aqueous NaOH 4 M until pH ≈ 10 and extracted with ethyl acetate (3 x 10 mL). The organic phases were combined, dried with Na_2SO_4 and the solvent was removed under reduced pressure.

Enantiopure amine (*R*)-**2c** was isolated as colorless liquid in 68% yield. For (*R*)-**2c** in >99% *ee*: $[\alpha]_D^{20}$ = +10.8 (c 0.5, $CHCl_3$); lit. $[\alpha]_D^{20}$ = -9.1 (c 0.5, $CHCl_3$) for (*S*)-**2c** in 98% *ee*].[16b]

ASSOCIATED CONTENT

Supporting Information.

Information about the transaminases employed in this study, details of the computational calculations, the chemical synthesis of the starting and reference materials including the NMR data of the products, the specific data of the conversions depicted in Figures 2 and 3, the analytics including the chromatograms, as well as the NMR spectra for described organic compounds. This material is available free of charge via the Internet at http://pubs.acs.org

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ACKNOWLEDGMENT

Financial support of this work by the Spanish Ministerio de Economía y Competitividad (CTQ-2013-44153-P project) and the Asturian Regional Government (FC-15-GRUPIN14-002) are gratefully acknowledged. M.L.-I. and D.G.-M. thank FICYT for a predoctoral fellowship. E. B.

thanks the European Union for a Marie Curie IEF grant (Project IEF-2011-BIOCASCADE). We thank R. Soria-Martínez for the assistance in computational studies.

ABBREVIATIONS

TA, transaminase; *E. coli*, *Escherichia coli*; Vf, *Vibrio fluvialis*; Cv, *Chromobacterium violaceum*; ArR, *Arthorbacter* species; At, *Aspergillus terreus*; AlaDH, alanine dehydrogenase; FDH, formate dehydrogenase; TLC, thin layer chromatography.

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