Transaminases Applied to the Synthesis of High Added-Value Enantiopure Amines

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Abstract. Critical parameters affecting the stereoselective amination of (hetero)aromatic ketones using transaminases have been studied such as temperature, pH, substrate concentration, co-solvent and source and percentage of amino donor, to further optimize the production of enantiopure amines using both (*S*)- and (*R*)-selective biocatalysts from commercial suppliers. Interesting enantiopure amino building blocks have been obtained overcoming some limitations of traditional chemical synthetic methods. Representative processes were scaled-up affording halogenated and heteroaromatic amines in enantiomerically pure form and good isolated yields.

1. Introduction

The synthesis of enantiopure amines is highly demanded due to the broad spectrum of biological activities that they display, but also due to their application as chiral building blocks

for the synthesis of more complex structures.[1] For instance, *ortho*- and *meta*-halogenated aromatic derivatives are intermediates of potent potassium channel openers (Figure 1a),[2] modulators of hypertension (Figure 1b),[3] calcimimetic agents to treat hyperparathyroidism (Figure 1c),[4] or anti-arthritic drugs.[5] Unfortunately, synthetic routes towards these compounds still remain challenging. Apart from the kinetic resolutions[6] of the racemic amines, where the maximum yield of the enantiopure product is 50%, some interesting examples involving sequential reactions[7] have recently been developed, but either the isolated yields or the selectivities were not completely satisfactory. A highly efficient system based on the ruthenium-catalyzed hydrogen transfer over sulfinylimines has also been described, obtaining excellent *ee* (98%) for the *meta*-chloro substituted compound, but *ee* decreased (91%) for the *ortho* derivative.[8]

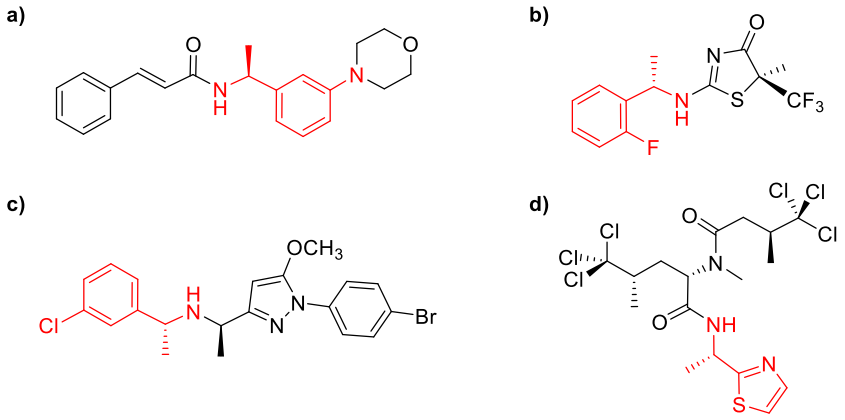


Figure 1. Examples of biologically active or natural compounds where halogenated aromatic or heteroaromatic chiral amines are key building blocks.

Another family of valuable amines includes those possessing a heteroaromatic ring close to the chiral amino group. Pyridine derivatives can be used as potential ligands or as acyl-transfer

catalysts to induce chirality.[9] Moreover, they have also been studied as building blocks for non-steroidal agents in the treatment of prostatic cancer.[10] On the other hand, the chiral 1-(thiazol-2-yl)ethylamine fragment is also present in natural compounds such as dysidenin (Figure 1d).[11] The selective synthesis of these amines has also proved to be difficult, and although several multi-step synthetic routes[11,12] and lipase-catalyzed resolutions of the corresponding racemates have been reported,[13] no general and straightforward methodologies for these chiral building blocks have been described yet.

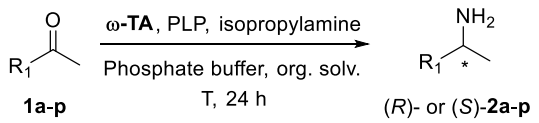
ω-Transaminases (ω-TAs, EC 2.6.1.x) have been extensively studied during the last few years for the synthesis of chiral amines, displaying excellent stereoselectivities under mild reaction conditions.[1a,14] Nevertheless, the application of ω-TAs to synthesize the target chiral amines **2b-p** (Scheme 1) has been scarcely developed.[15] Herein, several reaction parameters that affect the reductive amination processes of the commercially available ketone precursors for these amines were optimized, focusing on the substrate concentration for further scaling-up purposes.

2. Results and Discussion

In a first set of experiments, a screening kit of 24 commercially available transaminases was employed with acetophenone (**1a**, 25 mM) as a model substrate. The reactions were performed in phosphate buffer 100 mM pH 8 supplemented with pyridoxal phosphate (PLP, 0.25 mM) and isopropylamine (1 M)[16] or L- or D-alanine (50 mM) coupled with lactate dehydrogenase, glucose and glucose dehydrogenase to recycle the catalytic amount of the nicotinamide cofactor added in the medium,[17] thus shifting the thermodynamic equilibrium towards the desired amine formation. After this enzymatic screening (see Table S1 in the Supporting Information for further details), two (*S*)-selective, namely TA-P1-A06 and TA-P1-G06, and two (*R*)-selective transaminases,

namely ATA-025 and ATA-033, were chosen for further studies due to their highest activities (up to 54% conversion) and excellent selectivities (>99% *ee*). All four biocatalysts worked well under the very convenient isopropylamine approach, the last two transaminases being especially relevant since, apart from a few recent examples, not many ω-TAs have been found to display the (*R*)-stereopreference.[18] Having selected the adequate biocatalysts, the reaction conditions were studied to optimize the transamination process using ketones **1b**-**p** as starting materials (Scheme 1).

Scheme 1. Transaminase-catalyzed synthesis of enantiopure amines (*R*)- or (*S*)-**2a**-**p**.



1a-p (*R*)- or (*S*)-**2a-p**

a, R= Ph; **b**, R= *p*-F-C_6H_4; **c**, R= *p*-Cl-C_6H_4; **d**, R= *p*-Br-C_6H_4
e, R= *m*-F-C_6H_4; **f**, R= *m*-Cl-C_6H_4; **g**, R= *m*-Br-C_6H_4; **h**, R= *o*-F-C_6H_4
i, R= *o*-Cl-C_6H_4; **j**, R= *o*-Br-C_6H_4; **k**, R= *o*,*o*-diF-C_6H_3; **l**, R= *o*-Cl,*o*-F-C_6H_3
m, R= 4-Py; **n**, R= 3-Py; **o**, R= 2-Py; **p**, R= Thiazol-2-yl

The influence of an organic solvent applied to enzymatic transformations has previously been discussed in depth.[19] In the case of transaminases, although less developed, various examples recently showed that the use of an organic co-solvent can improve the enzymatic performance.[15f,18c,20] Thus, several solvents such as MeOH, EtOH, ^{i}PrOH, CH_3CN, tetrahydrofuran (THF) or dimethylsulfoxide (DMSO) were added at 2.5% v/v in the transformation of *p*-chloroacetophenone (**1c**) with ATA-033, demonstrating that their use was indeed beneficial, achieving higher activities with DMSO than when compared to the sole buffer as reaction medium (see Table S2 in the SI). Next, different proportions of DMSO were tested

without any further improvement (see Table S3 in the SI), therefore 2.5% v/v was selected as the optimum amount for subsequent reactions.

The pH profile of these transformations was also explored using *m*-bromoacetophenone (**1g**) as model substrate with ATA-025 and ATA-033, finding that a pH at around 7.5 was the most appropriate for the production of enantiopure (*R*)-**2g**, with up to 86% conversion (see Table S4 in the SI). A temperature study of the process was also performed and showed that 30 ºC was the optimum temperature for these reactions (see Table S5 in the SI). Finally, another amino donor, *n*-butylamine, was tested to replace isopropylamine with these biocatalysts, but only negligible conversions were achieved (see Table S6 in the SI).

Having in hand the best reaction conditions, ketones **1a**-**p** were transformed with these four transaminases obtaining the following results shown in Table 1. In the case of the halogenated derivatives, when compared with acetophenone (**1a**, entry 1), *para*-substituted compounds **1b**-**d** did not deter the enzymatic activity, obtaining slightly better results with the chlorinated and brominated ketones (entries 3 and 4) than with the fluorinated one (entry 2). This tendency was confirmed for the *meta*-halogenated derivatives **1e**-**g**, although higher conversions (60-85%) were achieved (entries 5-7). Surprisingly, sterically hindered *ortho*-substituted ketones **1h**-**j** (entries 8-10) afforded excellent conversions (>90%), in contrast to the results obtained with previous methodologies. 2-Fluoroacetophenone (**1h**) showed especially high conversions with all four biocatalysts, while chlorinated (**1i**) and brominated (**1j**) acetophenones were too bulky for the (*S*)-selective TAs, but not for the (*R*)-selective, obtaining quantitative conversions in this case. Pleasingly, all the amines were obtained with excellent *ee* (>97%).

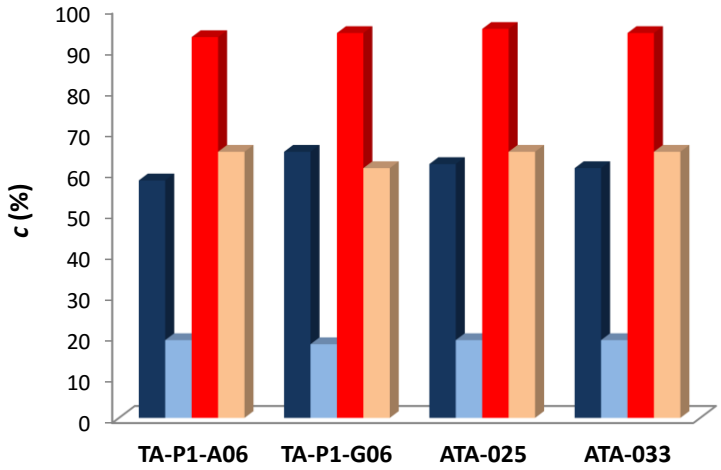


Figure 2. Positive influence of the halogen atom at *ortho* position in the TA-catalyzed reactions of **1h** (red bars) *vs* **1a** (blue bars) using 1 M (dark color) or 100 mM (light color) concentration of isopropylamine as amino donor.

These excellent (and unexpected) results towards the formation of the *ortho*-aromatic amines suggested that another effect could be present. Recently, Mutti and Kroutil have described a positive influence in the TA-catalyzed reactions over β-keto esters and β-oxygenated ketones due to the establishment of a likely intramolecular H-bond between the formed amine and the oxygen atom,[20b] therefore stabilizing the final compound and driving the transamination more easily. In our case, the formation of a stabilizing interaction between the amine and the halogen atom at the *ortho*-position could also be possible.[21] In order to demonstrate this effect, ω-TA-catalyzed reactions with acetophenone (**1a**) and *ortho*-fluoroacetophenone (**1h**) were performed separately with all four enzymes in the presence of a lower concentration of isopropylamine (100 mM) as amino donor, only a 4-fold excess with respect to the ketone substrate. As can be seen in Figure 2, while conversions for **1a** decreased from around 60% to less than 20% (3-fold), in the

case of **1h** conversions declined from 95% to 65% (1.5-fold), demonstrating a more favored process in the case of this ketone.

Table 1. Enzymatic asymmetric amination of ketones **1a-p** using ω-TAs and isopropylamine.[a]

entry	ketone	**TA-P1-A06**		**TA-P1-G06**		**ATA-025**		**ATA-033**	
		c (%)[b]	*ee* (%)[c]	*c* (%)[b]	*ee* (%)[c]	*c* (%)[b]	*ee* (%)[c]	*c* (%)[b]	*ee* (%)[c]
1	**1a**	58	99 (*S*)	65	99 (*S*)	62	99 (*R*)	61	99 (*R*)
2	**1b**	43	>99 (*S*)	43	>99 (*S*)	45	>99 (*R*)	43	>99 (*R*)
3	**1c**	53	98 (*S*)	58	>99 (*S*)	67	>99 (*R*)	60	>99 (*R*)
4	**1d**	52	>99 (*S*)	56	>99 (*S*)	63	99 (*R*)	58	98 (*R*)
5	**1e**	63	>99 (*S*)	63	>99 (*S*)	70	>99 (*R*)	71	>99 (*R*)
6	**1f**	70	>99 (*S*)	70	>99 (*S*)	74	>99 (*R*)	76	>99 (*R*)
7	**1g**	78	>99 (*S*)	73	>99 (*S*)	84	>99 (*R*)	86	>99 (*R*)
8	**1h**	93	>99 (*S*)	94	>99 (*S*)	95	>99 (*R*)	94	99 (*R*)
9	**1i**	64	98 (*S*)	48	99 (*S*)	98	>99 (*R*)	98	>99 (*R*)
10	**1j**	27	99 (*S*)	11	93 (*S*)	99	>99 (*R*)	99	>99 (*R*)
11	**1k**	83	>99 (*S*)	98	>99 (*S*)	99	>99 (*R*)	98	>99 (*R*)
12	**1l**	<1	n.d.	<1	n.d.	<1	n.d.	<1	n.d.
13	**1m**	87	89 (*S*)	93	90 (*S*)	83	87 (*R*)	89	88 (*R*)
14[d]	**1n**	41	>99 (*S*)	29	98 (*S*)	44	>99 (*R*)	42	96 (*R*)
15	**1o**	92	>99 (*S*)	92	>99 (*S*)	93	>99 (*R*)	92	>99 (*R*)
16[d]	**1p**	79	98 (*S*)	76	99 (*S*)	80	>99 (*R*)	83	>99 (*R*)

[a] Reaction conditions: Ketone **1a-p** (25 mM) in phosphate buffer 100 mM pH 7.5 with PLP (0.25 mM), TA (2 mg), DMSO (2.5% v/v), and isopropylamine (1 M) for 24 h at 30 ºC and 250 rpm. [b] Measured by GC. [c] Measured by chiral GC or HPLC. [d] Formation of 2-3% of a by-product.

We also exploited this effect to perform the successful transamination of the *ortho*,*ortho*-difluorinated ketone **1k** (entry 11) with complete conversions using three different enzymes, while unfortunately the dichlorinated substrate **1l** (entry 12) was already too bulky for these TAs.

Additionally, a series of heterocyclic ketones (**1m**-**p**) were tested with the four best transaminases to study the influence of a nitrogen atom in the aromatic ring, and then a 5-membered heteroaromatic ring such as thiazole. For the pyridinic compounds, when the nitrogen atom was at the 4- (**1m**, entry 13) or 2-position (**1o**, entry 15), conversions were excellent, although selectivity decreased for substrate **1m**. In the case of derivative **1n**, a much lower conversion was achieved (29-44%, entry 14). In the case of the thiazole-derived ketone **1p**, high conversions (76-83%) of the enantioenriched amine were obtained (>97% *ee*, entry 16).

Several selected transamination reactions were scaled-up to 200 mg with the corresponding ketone at a 100 mM substrate concentration (Table 2), obtaining high to excellent conversions and complete selectivities with the *ortho*-halogenated ketones **1h**-**j** (entries 2-4), while moderate to high conversions were achieved with **1g** and **1p** (entries 1 and 5). Furthermore, additional experiments were performed at higher substrate concentration (250 mM) at a 250 mg-scale using only a tenth of the enzyme loading (20 mg), obtaining conversions higher than 85% into enantiopure brominated amine (*R*)-**2j** after 48 h employing ATA-025 and ATA-033 (entries 6 and 7).

Table 2. Scale-up in the transamination reactions of amines **1g-j**,**p**.[a]

entry	ketone	enzyme[b]	scale (mg)	[**1g-j**,**p**] (mM)	time (h)	*c* (%)[c]	*ee* (%)[d]
1	**1g**	TA-P1-A06 (100 mg)	200	100	24	74	99 (*S*)
2	**1h**	TA-P1-G06 (100 mg)	200	100	24	87	>99 (*S*)
3	**1i**	ATA-025 (100 mg)	200	100	24	96	>99 (*R*)
4	**1j**	ATA-033 (100 mg)	200	100	24	95	>99 (*R*)
5	**1p**	TA-P1-G06 (100 mg)	200	100	24	59	>99 (*S*)
6	**1j**	ATA-025 (20 mg)	250	250	48	85	>99 (*R*)
7	**1j**	ATA-033 (20 mg)	250	250	48	89	>99 (*R*)

[a] Reaction conditions: Ketone **1g-j**,**p** in phosphate buffer 100 mM pH 7.5 with PLP (0.25 mM), TA, DMSO (2.5% v/v), and isopropylamine (1 M) at 30 ºC and 250 rpm. [b] Source and loading of enzyme for the transformation. [c] Measured by GC. [d] Measured by chiral GC or HPLC.

Finally, in order to demonstrate the applicability of the process at a much higher scale, 1 g of ketones **1g-j** were subjected to biocatalytic transamination at a 100 mM substrate concentration, obtaining similar conversions to those achieved with 200 mg of substrate and recovering the enantiopure amines with high isolated yields after a simple extraction protocol (Figure 3).

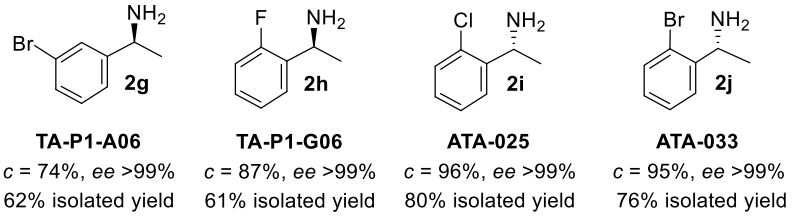


Figure 3. Results from the biocatalytic transamination of selected ketones at 1 g-scale and 100 mM substrate concentration.

3. Conclusions

In summary, we have demonstrated the use of transaminases as a potent tool applied for the synthesis of a series of enantiopure amines whose chemical routes were previously based on multi-step protocols or did not afford the final targets with satisfactory selectivities. After a careful review of the literature, we have found that described chemo- or enzymatic methods allow the production of (*S*)-**2g** in >99% *ee* and 47% isolated yield through lipase-catalyzed resolution of the racemic amine,[6b] or alternatively in 62% yield with 71% *ee* under organocatalytic transamination.[7a] Also under these conditions (*S*)-**2h** could be synthesized with a 65% isolated yield and 84% *ee*,[7a] while employing a sequential amination-hydrogenolysis protocol, it was obtained in 44% yield and 98% *ee*.[7b] Amine (*R*)-**2i** was isolated through asymmetric transfer hydrogenation of a sulfonylimine precursor with 96% yield and 91% *ee*.[8a] On the other hand, there is just one example described by Truppo and co-workers where (*R*)-**2j** was synthesized via transaminase-catalyzed reaction in a 50% isolated yield with >98% *ee*.[15f]

Herein, starting from easily available ketones and after optimization of the enzymatic reaction conditions, it has been demonstrated that several commercially available TAs can be employed to obtain various halogenated and heterocyclic amines. Efforts have been focused on the optimization of the scale-up of the biocatalytic transamination and the substrate concentration, so taking into account other TA-catalyzed protocols, the transformations were successfully carried out at higher concentrations between 100-250 mM (14-50 g/L), affording in all cases the enantiopure amines with high isolated yields. Thus, this optimization study clearly offers a new advantageous alternative in terms of production and economic issues towards derivatives which are not easily accessible.

Experimental Section

General. Ketones **1a**-**p** and amines **2a**,**d**,**m**-**o** were purchased from commercial sources and used as received. Codex® Transaminase Screening Kit (ATASK-000250), PLP, lactate dehydrogenase and glucose dehydrogenase present in the PRM-102 mix were purchased from Codexis. All other reagents and solvents were of the highest purity available.

Chemical reactions were monitored by analytical TLC, performed on silica gel 60 F254 plates and visualized by UV. Flash chromatography was performed using silica gel 60 (230-400 mesh). IR spectra were recorded on an infrared Fourier transform spectrophotometer on NaCl pellets. NMR spectra were recorded at 300 (^{1}H), and 75 (^{13}C) MHz. The chemical shifts (δ) are reported in parts per million (ppm) relative to the solvent residual signals and the coupling constants (*J*) in Hertz (Hz). ESI-TOF mode was used to record high resolution mass spectra (HRMS). Gas chromatography (GC) analyses were performed on a standard gas chromatograph equipped with a FID. HPLC analyses were performed using a standard HPLC chromatograph with UV detection. Optical rotations were measured using a standard polarimeter with a sodium lamp (D) and are reported in units of 10^{-1} deg cm^2 g^{-1}.

General protocol for the TA-catalyzed amination of ketones 1a-p. In a 1.5 mL Eppendorf tube, **1a-p** (25 mM) was dissolved in DMSO (12 μL, 2.5% v/v) and phosphate buffer 100 mM pH 7.5 (485 μL, 0.25 mM PLP, 1 M isopropylamine) and the corresponding transaminase (2 mg) were added. The reaction was shaken at 30 ºC and 250 rpm for 24 h and stopped by addition of 10 N NaOH (400 μL). Then the mixture was extracted with ethyl acetate (2 × 500 μL), the organic layers were separated by centrifugation (90 sec, 13000 rpm), combined and finally dried over Na_2SO_4. Conversions of **2a**-**p** were determined by GC. In most cases, acetylation of the sample was necessary to measure *ee*. For results, see Table 1 in the manuscript.

Scale-up of the transamination reactions. In a 250 mL Erlenmeyer flask, **1g-j** (1 g, 100 mM) was dissolved in DMSO (2.5% v/v) and phosphate buffer 100 mM pH 7.5 (0.25 mM PLP, 1 M isopropylamine) and the corresponding transaminase (400 mg) were added. The reaction was shaken at 30 ºC and 250 rpm for 24 h and stopped by addition of 6 N HCl until acid pH is achieved. Before that, a 200 µL aliquot was taken and treated as previously described to measure conversion and enantiomeric excess of the product. Then, the reaction mixture was filtered and the unreacted ketone and DMSO separated by continuous extraction using CH_2Cl_2 (80 mL) for 72 h. Next, the aqueous phase was basified until pH 10 with NaOH pellets and extracted with CH_2Cl_2 (3 × 50 mL). Organic phases were combined, dried over Na_2SO_4, and the solvent evaporated under reduced pressure to finally obtain the pure amine. Isolated yields: 62% (**2g**), 61% (**2h**), 80% (**2i**) and 76% (**2j**).

Supporting Information. Experimental procedures for the chemical synthesis of racemic amines and the biocatalytic transamination experiments, full characterization of novel compounds, spectroscopic and analytical data of the enantioenriched final products are included.

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Notes

The authors declare no competing financial interest.

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