Catalytic Promiscuity of Transaminases: Preparation of Enantioenriched β-Fluoroamines *via* Formal Tandem Hydrodefluorination-Deamination

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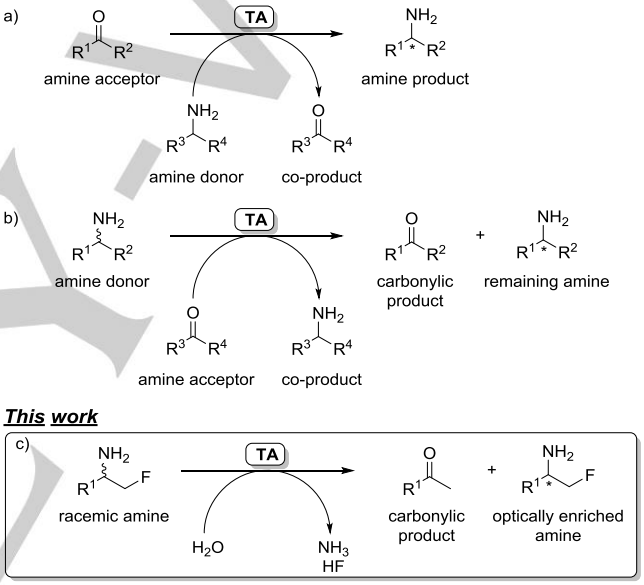
Abstract: Transaminases are valuable enzymes for industrial biocatalysis that enable the preparation of optically pure amines. For these transformations they require either an amine donor (amination of ketones) or an amine acceptor (deamination of racemic amines). In this work transaminases are shown to react with aromatic β-fluoroamines, leading to simultaneous enantioselective dehalogenation and deamination to form the corresponding acetophenone derivatives in the absence of an amine acceptor. A series of racemic β-fluoroamines was resolved in a kinetic resolution *via* tandem hydrodefluorination-deamination, giving the corresponding amines with up to >99% *ee*. This is a first example of exploiting the catalytic promiscuity of transaminases as a tool for novel transformations.

In recent years, many enzymes have been shown to efficiently catalyze transformations that appear far removed from their natural activity. This catalytic promiscuity[1] can be useful for synthetic purposes, broadening the applicability of biocatalysts. While many examples have recently been described for hydrolases,[2] the application of these unconventional processes for other enzyme classes has not been fully developed.[3] Among biocatalysts that have demonstrated broad applicability, transaminases (TAs) can be highlighted, as they perform the amination of an amine acceptor (ketone or aldehyde) using an amine donor (*e.g.*, alanine or isopropylamine, Scheme 1a), mediated by the cofactor pyridoxal 5'-phosphate (PLP).[4,5] Interestingly, transaminases can also catalyze the reverse reaction, achieving the kinetic resolution of racemic amines *via* amination of an amine acceptor (*e.g.*, pyruvate or acetone, Scheme 1b). This process is hampered by the disadvantage of a maximum of 50% yield, but it is thermodynamically favored in comparison to the amination route.[4,6]



Scheme 1. Methodologies to obtain enantiopure amines using TAs by: a) amination of a carbonyl compound using an amine donor; b) deamination of a racemic amine employing an amine acceptor; and c) novel tandem hydrodefluorination-deamination kinetic resolution of racemic β-fluoroamines.

The introduction of fluorine atom(s) to an organic derivative has a significant influence on, *e.g.* their physicochemical, conformational and metabolic properties.[7] The cleavage of a C−F into a C−H bond, hydrodefluorination,[8] has been performed in the case of aromatic derivatives using transition metal complexes under harsh reaction conditions. For aliphatic fluorinated compounds however, alternatives still remain to be found,[9] partly due to the inertness of C−F bonds.[10] β-Fluoroamines are potent inhibitors of PLP-dependent enzymes such as aminotransferases and decarboxylases.[11] These proteins are crucial in living organisms and are therefore commonly envisaged as therapeutic targets.[12] Silverman and co-workers demonstrated that 4-amino-5-fluoropentanoic acid was an irreversible inhibitor of γ-aminobutyrate aminotransferase (GABA-AT).[13]

Herein we show that a promiscuous reactivity of transaminases can be applied to obtain a series of enantiopure

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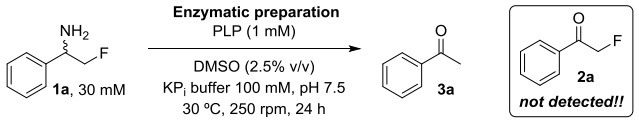
β-fluoroamines by unprecedented formal *tandem hydrodefluorination-deamination kinetic resolution*, starting from the racemic β-fluoroamines, in the *absence* of an amine acceptor, under simple and mild conditions in aqueous medium (Scheme 1c). Thus, it was exploited that the inhibition of certain transaminases by β-fluoroamines is negligible under the reaction conditions employed.

Table 1. Enzymatic transformations on β-fluoroamine **1a**.[a]

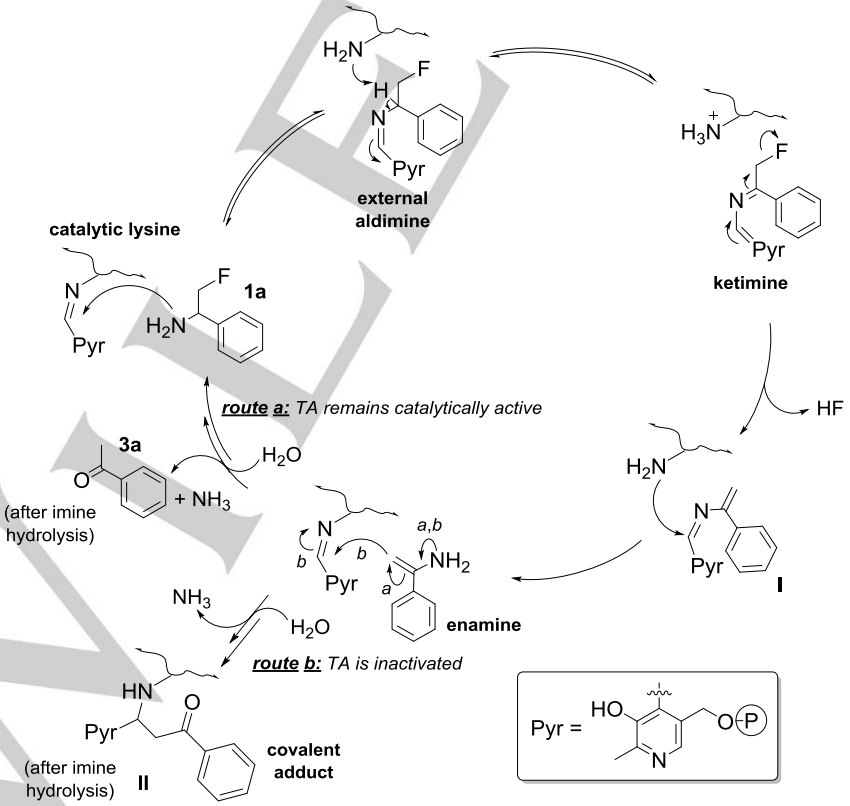


Entry	Enzymatic preparation	**1a** [%][b]	**2a** [%][b]	**3a** [%][b]
1	--	>99	<1	<1
2	ATA-231[c]	41	<1	59
3	TA-P1-F03[c]	53	<1	47
4	TA-P1-G06[c]	28	<1	72
5	BSA	>99	<1	<1
6	CAL-B	>99	<1	<1
7	Lyo. *E. coli*	>99	<1	<1

[a] For reaction conditions, see Supporting Information. [b] Measured by GC analysis. [c] Transaminase commercially available from Codexis company.

To study the reactivity of TAs with fluorinated compounds,[5c,14] we focused on the most thermodynamically favored reaction conditions using β-fluoroamine **1a** and acetone as amine acceptor, in order to obtain the corresponding enantioenriched amine **1a**, together with α-fluoro ketone **2a** under typical kinetic resolution conditions (Scheme 1b). Several commercially available TAs did not afford this product but acetophenone **3a** to a high extent (data not shown). Furthermore, similar conversions were attained in the absence of the amine acceptor (Table 1), with only the dehalogenated ketone **3a** detected as a product (entries 2-4). Since the substrate was stable in the blank reaction (entry 1), and in the presence of other enzymatic or protein preparations such as bovine serine albumin (BSA), lipase B from *Candida antarctica* (CAL-B) and lyophilized cells of *E. coli* (entries 5-7), it was clear that this amine was transformed by the tested TAs, resulting in both hydrodefluorination and deamination reactions.

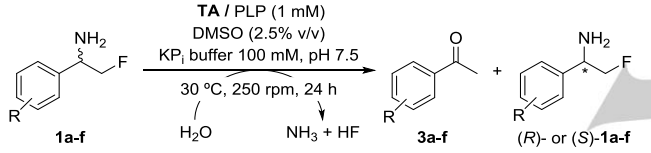
Further experiments also demonstrated that this transformation may occur in hand with enzymatic deactivation, as in the presence of fluorinated substrates, selected TAs lost their activity at least partially (see Supporting Information for more details). Our next objective was the proposal of a plausible mechanism that could explain these results. Based on the work by Silverman and co-workers on GABA-AT inhibitors,[14] and with reference to his previous mechanistic studies,[15,16] we suggest a dual mechanism for defluorination and inhibition (Scheme 2).



Scheme 2. Proposed dehalogenation (route *a*) and inhibition (route *b*) mechanisms catalyzed by TAs employing β-fluoroamine **1a**.

As a first step, PLP, which is linked to the catalytic lysine of the TA,[17] is transferred to the reacting amine **1a** forming the external aldimine intermediate. Then, it rearranges into the ketimine intermediate, which is converted into the dehalogenated aldimine **I** due to the presence of the fluorine atom at the α-position acting as a leaving group. Then, the catalytic lysine can attack the imine bond generating the internal aldimine together with an enamine derivative. At this stage, two different pathways can occur. If the enamine quickly diffuses into the reaction medium (route *a*), it will be hydrolyzed affording the defluorinated ketone (in this case **3a**), and the enzyme will be able to start a new catalytic cycle, therefore *not having been inactivated*. However, if the enamine remains in the active site of the enzyme for a sufficient time (route *b*), it will attack the internal aldimine created by PLP and the catalytic lysine, thus providing an imine which, after hydrolysis, will furnish a final covalent adduct (**II**) *inactivating the transaminase irreversibly*. Moreover, the proposed mechanism can explain the reason why an amine acceptor is not necessary, as the transfer of an amine group does not occur, rather it is a simple hydrolysis of an imine bond releasing an ammonia molecule.

Table 2. Tandem hydrodefluorination-deamination kinetic resolution of β-fluoroamines **1a**-**f**.[a]



a, R= H; **b**, R= *p*-F; **c**, R= *p*-Cl; **d**, R= *p*-Br; **e**, R= *m*-OMe; **f**, R= *p*-OMe

Entry	Substrate	CV-TA		ArR-TA	
		3a-**f** [%][b]	*ee* **1a**-**f** [%][c,d]	**3a**-**f** [%][b]	*ee* **1a**-**f** [%][c,d]
1	**1a**	53	99 (*S*)	22	23 (*R*)
2	**1b**	50	99 (*S*)	67	>99 (*R*)
3	**1c**	52	>99 (*S*)	51	>99 (*R*)
4	**1d**	51	>99 (*S*)	28	40 (*R*)
5	**1e**	60	>99 (*S*)	54	>99 (*R*)
6	**1f**	55	99 (*S*)	55	>99 (*R*)

[a] For reaction conditions, see Supporting Information. [b] Measured by GC analysis. [c] Measured by chiral GC analysis. [d] Change in Cahn-Ingold-Prelog (CIP) priority.

Then, it was investigated if this reaction could be performed enantioselectively. Ideally, a kinetic resolution *via* tandem hydrodefluorination-deamination could be achieved (Scheme 1c). A series of transaminases overexpressed in *E. coli* was employed: the *S*-selective TA from *Chromobacterium violaceum* (CV-TA),[18] *S*- and *R*-selective TAs from *Arthrobacter* sp. (ArS-TA[19] and ArR-TA,[20] respectively), and a variant of the ArR-TA with mutations in 27 positions (ArRmut11-TA),[5c] which is also *R*-selective. These TAs have displayed excellent activities for aromatic substrates, providing the corresponding amines with high selectivities.[21] A set of racemic aromatic β-fluoroamines (**1a**-**f**) was purchased or synthesized as described in the SI.[22]

We used lyophilized preparations of *E. coli* cells due to their easy handling and high stability.[21c,d] After a first enzymatic screen (see SI), we obtained the best results for CV-TA and ArR-TA, resulting in excellent resolutions for most of the substrates (Table 2). Thus, different derivatives, including those with electron-donating or withdrawing groups at the phenyl moiety, were obtained with high enantiomeric excess. Moreover, depending on the transaminase used, both enantiomers were attained either in optically pure form or at least optically enriched. Thus, CV-TA reacted preferentially with the (*R*)-antipode and ArR-TA with the (*S*)-enantiomer.

Finally, we applied this transformation concept on a preparative scale. Thus, 100 mg of racemic amines **1a** and **1d** were resolved using CV-TA into the corresponding (*S*)-derivatives (>99% *ee*) within 32 h, and with 75-80% yield of isolated product after a simple extraction protocol (see SI).

In order to obtain evidence of the mechanism of inhibition proposed in Scheme 2, the X-ray crystal structure of ArRmut11-TA was obtained from protein that had been pre-incubated with amine **1d**. Details of the structure can be found in the Supporting Information. Following building and refinement of protein and water atoms, clear electron density was observed in the omit map at the interface of six dimer pairs for PLP (corresponding to twelve PLP sites). Two regions of continuous electron density projected from the electrophilic carbon of PLP: the first connecting the PLP to the side-chain of Lys188, and the second extending from the PLP into the active site. This density (Figure 1) was successfully modelled as the adduct resulting from inhibition of the enzyme by **1d**, representing structure **II** in Scheme 2.

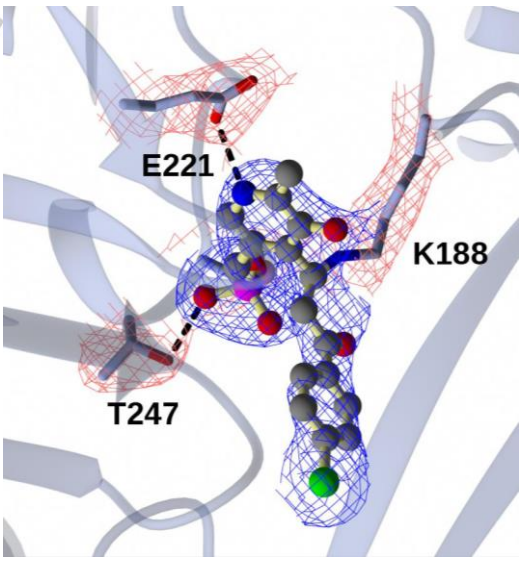


Figure 1. Active site at the dimer interface of ArRmut11-TA inhibited by complexation with **1d** and showing PLP covalently bonded to both K188 and the residual ligand, representing complex **II** as shown in Scheme 2. Backbone and C-atoms of subunit 'A' are shown in light blue. PLP adduct is shown in ball-and-stick format with carbon atoms in grey and the bromine atom in green. Electron density maps correspond to the F_o-F_c omit map and the $2F_o$-F_c map in blue and red, and at levels of 3σ and 1σ respectively. The omit map is that obtained prior to modelling and refinement of the ligand.

In summary, we have reported a novel enantioselective transformation based on a promiscuous activity of transaminases, namely a tandem hydrodefluorination-deamination reaction in aqueous medium under very mild conditions. This process was demonstrated for the kinetic resolution of racemic β-fluoroamines to obtain the non-converted amines with excellent *ee*. Remarkably no amine acceptor such as, *e.g.* acetone, was necessary. Depending on the biocatalyst of choice, both enantiomers could be obtained. Hydrodefluorination is an interesting reaction that has been studied during recent years.[8] The use of metal complexes has allowed the cleavage of aromatic C–F bonds, but methods to cleave aliphatic bonds are not accessible. The proposed biocatalytic concept expands the toolkit for asymmetric synthesis complementing previous chemical processes, and illustrates the potential of catalytic promiscuity as a tool for designing unprecedented reactions.

Acknowledgements

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Keywords: transamination • catalytic promiscuity • biocatalysis • kinetic resolution • hydrodefluorination

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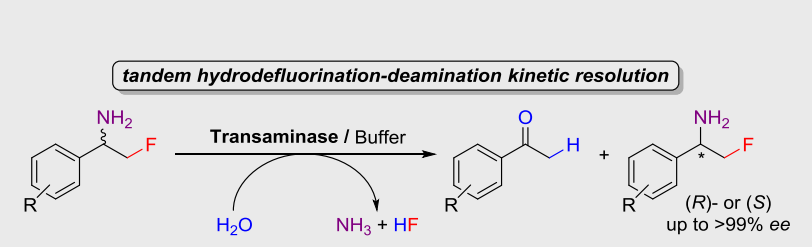

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