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Catalytic Promiscuity of Transaminases: Preparation of Enantioenriched β-Fluoroamines by Formal Tandem Hydrodefluorination/Deamination

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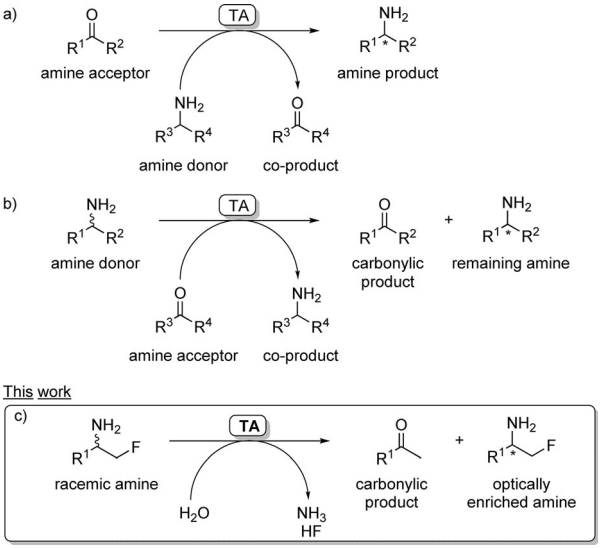
Abstract: *Transaminases are valuable enzymes for industrial biocatalysis and enable the preparation of optically pure amines. For these transformations they require either an amine donor (amination of ketones) or an amine acceptor (deamination of racemic amines). Herein transaminases are shown to react with aromatic β-fluoroamines, thus 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 by tandem hydrodefluorination/deamination, thus giving the corresponding amines with up to greater than 99% ee. This protocol is the 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 by 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, thus achieving the kinetic resolution of racemic amines by 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, for example, 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 because of 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

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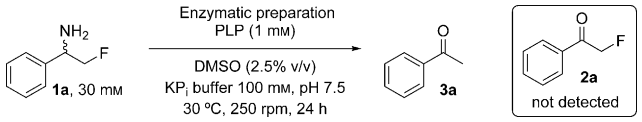
that 4-amino-5-fluoropentanoic acid was an irreversible inhibitor of γ-aminobutyrate aminotransferase (GABA-AT).[13]

Herein we show that the promiscuous reactivity of transaminases can be applied to obtain a series of enantiopure β-fluoroamines by an unprecedented formal tandem hydrodefluorination/deamination kinetic resolution of racemic β-fluoroamines. The reaction proceeds in the absence of an amine acceptor and under simple and mild reaction 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.

To study the reactivity of TAs with fluorinated compounds,[5c,14] we focused on the transformation of 2-fluoro-1-phenylethan-1-amine (**1a**) with acetone as the amine acceptor to obtain the remaining enantioenriched amine **1a**, together with transformed 2-fluoro-1-phenylethan-1-one (**2a**) under typical kinetic resolution conditions as generalized in Scheme 1b. Several commercially available TAs did not afford this product but acetophenone (**3a**) was produced (data not shown). Furthermore, similar conversions were attained in the absence of the amine acceptor (Table 1), with only

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

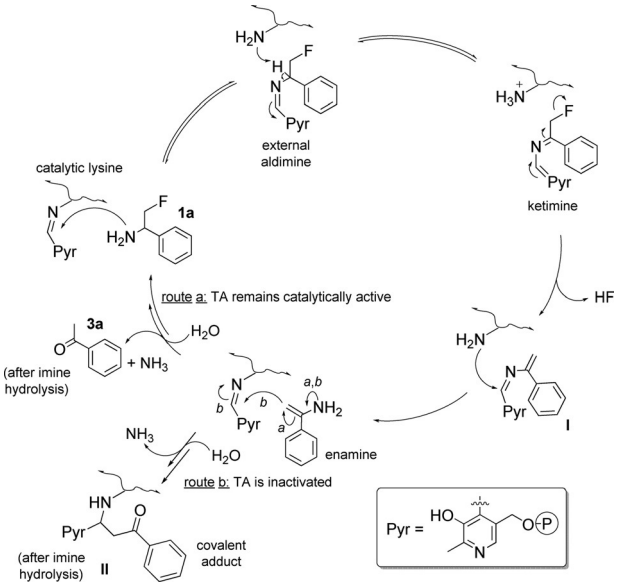


Entry	Enzymatic preparation	Conversion [%][b]		
		1a	**2a**	**3a**
1	–	>99	<1	<1
2	ATA-231[c]	41	<1	59
3	TA-P1-F03[c]	53	<1	47
4	TA-P1-G06[c]	28	<1	72
5	BSA	>99	<1	<1
6	CAL-B	>99	<1	<1
7	Lyo. *E. coli*	>99	<1	<1

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

acetophenone 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, thus resulting in both hydrodefluorination and deamination reactions.

Further experiments also demonstrated that this transformation may occur along with enzymatic deactivation because in the presence of fluorinated substrates, selected TAs lost their activity, at least partially (see the Supporting Information for more details). Our next objective was the proposal of a plausible mechanism which could explain these results. Based on the work by Silverman and co-workers on GABA-AT inhibitors,[13] and with reference to his previous



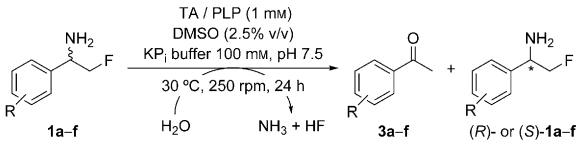
Scheme 2. Proposed dehalogenation (route a) and inhibition (route b) mechanisms as catalyzed by TAs employing the β-fluoroamine **1a**.

mechanistic studies,[15,16] we propose a dual mechanism for defluorination and inhibition (Scheme 2). As a first step, PLP, which is linked to the catalytic lysine of the TA,[17] is transferred to the reacting **1a**, thus forming the external aldimine intermediate. Then, it rearranges into the ketimine intermediate, which is converted into the dehalogenated aldimine **I** as a result of the fluorine atom serving as a leaving group. Then, the catalytic lysine can attack the imine bond to generate 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, thus affording the defluorinated ketone (**3a**), and the enzyme will be able to start a new catalytic cycle as it is not 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 to provide an imine which, after hydrolysis, will furnish a final covalent adduct (**II**), thus inactivating the transaminase irreversibly. Moreover, the proposed mechanism explains why an amine acceptor is not necessary, that is, the transfer of an amine group does not occur. Instead, it involves the hydrolysis of an imine bond to release a molecule of ammonia.

Then, it was investigated if this reaction could be performed enantioselectively. Ideally, a kinetic resolution by 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, thus providing the corresponding amines with high selectivities.[21]

Racemic aromatic β-fluoroamines (**1a–f;** Table 2) were either purchased or synthesized as described in the Supporting Information.[22] We used lyophilized preparations of

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



1a: R = H **1d**: R = *p*-Br
1b: R = *p*-F **1e**: R = *m*-OMe
1c: R = *p*-Cl **1f**: R = *p*-OMe

Entry	Substrate	CV-TA		ArR-TA	
		Conv. **3** [%][b]	*ee* **1** [%][c,d]	Conv. **3** [%][b]	*ee* **1** [%][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 the Supporting Information. [b] Measured by GC analysis. [c] Measured by GC analysis using a chiral stationary phase. [d] Change in Cahn–Ingold–Prelog (CIP) priority.

E. coli cells because of their easy handling and high stability.[21c,d] After screening the enzymes (see the Supporting Information), we obtained the best results for CV-TA and ArR-TA, thus resulting in excellent resolutions for most of the substrates. Therefore, different derivatives, including those with either electron-donating or electron-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 each of the racemic amines **1a** and **1d** were resolved using CV-TA into the corresponding *S*-configured derivatives (>99% *ee*) within 32 h, giving 75–80% isolated yield (referring to the single enantiomer) after a simple extraction protocol (see the Supporting Information).

To obtain evidence of the mechanism of inhibition proposed in Scheme 2, the X-ray crystal structure of ArRmut11-TA was obtained from a protein that had been pre-incubated with **1d**. Details of the structure can be found in the Supporting Information. After building and refinement of the 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 atom of PLP (Figure 1): the first region connected the PLP to the side-chain of Lys188, and the second extended from the PLP into the active site. This density was successfully modelled as the adduct resulting from inhibition of the enzyme by **1d**, thus representing the structure **II** in Scheme 2.

In summary, we have reported a novel enantioselective transformation based on the promiscuous activity of transaminases, namely a tandem hydrodefluorination/deamination reaction in aqueous medium under very mild reaction

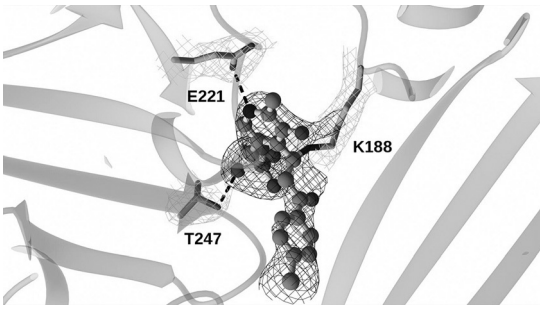


Figure 1. Active site at the dimer interface of ArRmut11-TA inhibited by complexation with **1d** and showing PLP covalently bonded to both K188 and the residual ligand, thus representing complex **II** as shown in Scheme 2. The PLP adduct is shown in ball-and-stick format. Electron density maps correspond to the F_o-F_c omit map and the $2F_o-F_c$ map in black and gray meshing, respectively, and at the corresponding levels of 3σ and 1σ. The omit map is that obtained prior to modelling and refinement of the ligand.

conditions. This process was demonstrated for the kinetic resolution of racemic β-fluoroamines to obtain the non-converted amines with excellent *ee* values. Remarkably no amine acceptor, such as 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.

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