COMMUNICATION

Transaminases as suitable catalysts for the synthesis of enantiopure β,β-difluoroamines

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Transaminases have demonstrated the ability to catalyze the amination of a series of aliphatic and (hetero)aromatic α,α-difluorinated ketones with high stereoselectivity, thus providing the corresponding β,β-difluoroamines in high isolated yields (55-82%) and excellent enantiomeric excess (>99%). It was also observed that these activated substrates could be quantitatively transformed employing a small molar excess of the amine donor since this amination processes were thermodynamically favored. Selected transformations could be scaled up to 500 mg, showing the robustness of this methodology.

The introduction of the CF_2H group in a molecule confers it with special properties since this moiety is weakly acidic and thus can perform hydrogen bonds with other groups, although it is less hydrophilic than alcohols or thiols.[1] Therefore, it is common that compounds bearing this moiety present interesting biological properties, and this fact has been reflected in the efforts made by chemists in order to design novel synthetic approaches to reach these types of substrates.[2] However, difluoromethylation protocols are underdeveloped compared to, *e.g.* monofluoro- or trifluoromethylation reactions, especially if stereoselectivity must also be introduced.[1a,2b]

This is also true for β,β-difluoroamines ($H_2NCR^1R^2–CF_2H$), which can be considered as bioisosters of 1,2-aminoalcohols.[1c,1d,2a] While some excellent chemical methods have been described to obtain these compounds in a non-stereoselective fashion,[3] the synthesis of the enantioenriched derivatives remains as a challenge (Fig. 1, top). Hence, Pd-catalyzed hydrogenation[4] or organocatalyzed transfer hydrogenation[5] of difluorinated N-protected ketimines, the addition of acetone mediated by proline,[6] silyl dienolates catalyzed by a Lewis acid,[7] or a chiral carbanion[8] over fluorinated N-protected aldimines have been described as suitable methods to access enantioenriched β,β-difluoroamines. On the other hand, these compounds have been accessed through multi-step synthetic routes involving, *e.g.* the addition of difluoromethyl phenyl sulfone on a chiral sulfinylimine[9] or the formation of a diastereomeric mixture of a 2-difluoromethyloxazolidine.[10] Also, the organocatalyzed addition of fluoride over activated alkenes[11] or *N*-2,2-difluoroethylthioisatin ketimines on nitroalkenes[12] have demonstrated to be useful approaches in the field. Finally, the lipase-catalyzed alcoholysis of racemic N-acylated 2,2-difluoro-1-phenylethan-1-amine *via* kinetic resolution[13] has also been described.

Unfortunately, most of these methodologies have been applied for a specific substrate,[6,13] providing the final product(s) with moderate enantioselectivities, and/or making use of various steps[8,10,12] –thus lowering the overall yield–, including the deprotection of an N-protecting group, which sometimes could not be easily removed.[4,5] These protocols were performed in organic medium and in some cases at high (or very low)[7,9,11] temperature using harmful or unstable reagents; therefore, a general alternative in aqueous medium under mild reaction conditions seems to be highly appealing.

At first glance, a direct approach to obtain these compounds would be the reductive amination of the corresponding α,α-difluoroketones, precursors which are easily accessible,[14] using a chiral catalyst. However, this transformation on such activated carbonyl compounds generally present low selectivities due to the concomitant formation of the alcohol derivatives by direct attack of the hydride to the highly electrophilic ketone moiety. In fact, when we studied the action of different reductive aminases (RedAms)[15] on a series of fluorinated substrates,[16] it was observed that they showed a promiscuous carbonyl reductase activity,[17] affording mixtures of the chiral alcohols and primary or secondary amines at variable proportions depending on the substitution pattern at the α position of the ketone and the amine used as donor (Fig. 1, bottom). Thus, in the case of α,α-difluoroketones, the major product was usually the alcohol derivative.[16]

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Electronic Supplementary Information (ESI) available: characterization of synthesized compounds, enzymatic screenings and protocols, analytical data, chromatograms and NMR spectra are included. See DOI: 10.1039/x0xx00000x

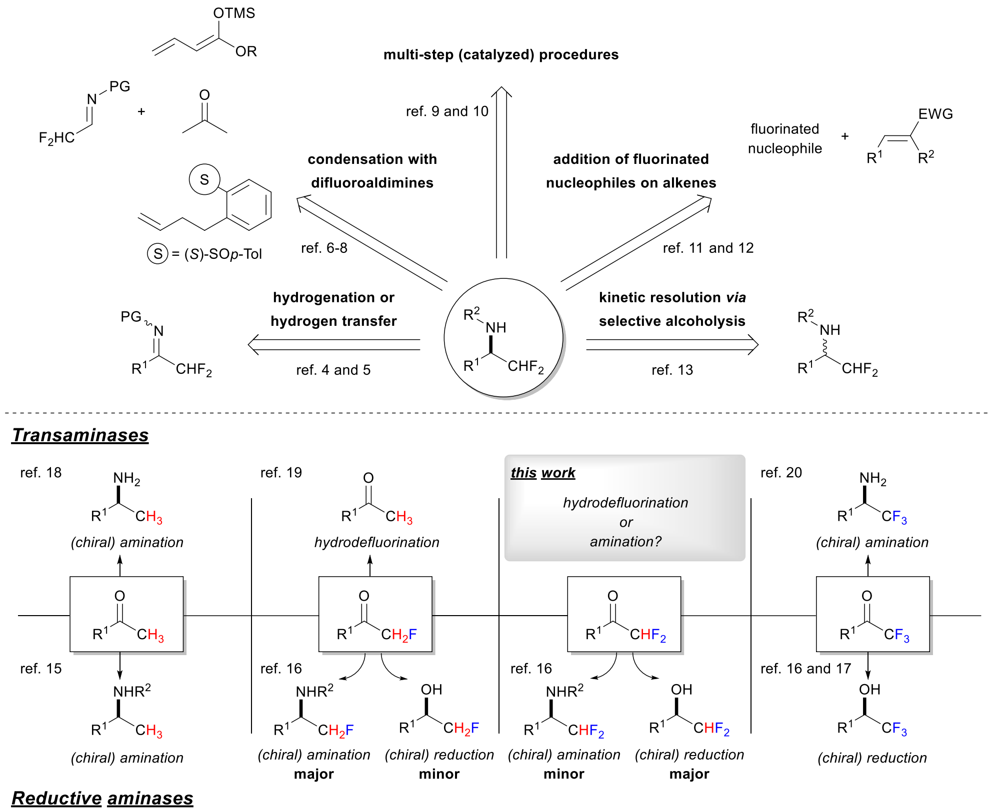
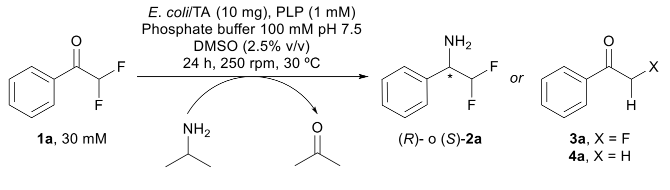


Fig. 1 Synthetic approaches to obtain enantioenriched β,β-difluoroamines (top) and reactivity of (fluorinated) ketones with transaminases and reductive aminases (bottom).

Pyridoxal 5′-phosphate (PLP)-dependent transaminases (TAs)[18] have also shown a particular reactivity with these substrates, displaying hydrodefluorinase activity (substitution of a C–F bond by a C–H bond) when reacting with α-fluoroketones,[19] while the trifluorinated analogs are converted into the expected (chiral) fluorinated amines (Fig. 1, bottom).[20]

Table 1 Reactivity of 2,2-difluoro-1-phenylethan-1-one (**1a**) with transaminases[a]



Entry	TA	[2-PrNH$_2$] (mM)	**1a** (%)[b]	**2a** (%)[b]	***ee* 2a** (%)[c,d]	**3a** (%)[b]	**4a** (%)[b]
1[e]	-	1000	>99	<1	n.d.	<1	<1
2[f]	ArR	50	<1	98	>99 (*S*)	<1	2
3	ArR	100	<1	>99	>99 (*S*)	<1	<1
4	ArR	1000	63	37	>99 (*S*)	<1	<1
5	CV	100	30	70	>99 (*R*)	<1	<1
6	ArS	100	73	27	86 (*R*)	<1	<1
7	ArRmut11	1000	<1	99	>99 (*S*)	1	<1

[a] For experimental details, see ESI. [b] Substrate and product percentages measured by GC. [c] Enantiomeric excess values measured by chiral GC. [d] Change in the CIP priority. [e] Blank reaction without enzyme. [f] 20 mM of **1a**. n.d. not determined.

Therefore, we found of interest to study the behavior of a panel of α,α-difluoroketones as possible substrates of a set of transaminases and compare their reactivity with the monofluorinated and trifluorinated counterparts, this is, *would they be defluorinated or (stereoselectively) aminated*?

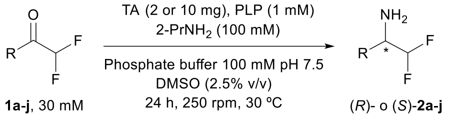
To answer this question, commercially available 2,2-difluoro-1-phenylethan-1-one (**1a**, 30 mM) was tested with a series of heterologously expressed TAs in *E. coli* under two different conditions: (*a*) employing a huge molar excess of 2-propylamine (2-PrNH$_2$, 1 M) used as amine donor to favor the amination reaction,[18] and (*b*) applying a small molar excess of 2-PrNH$_2$ (100 mM) with respect to the substrate to achieve the dehalogenation reaction, since it is necessary just 1 equivalent of the amine to eliminate one fluorine atom[19] (in **1a** there are two, so the minimum amine concentration to obtain acetophenone (**4a**) would be 60 mM). The reactions were performed under typical conditions, adding phosphate buffer 100 mM pH 7.5 and dimethylsulfoxide (DMSO, 2.5% v/v) to solubilize the substrate, at 30 °C and under orbital shaking (250 rpm). Due to easy handling and high stability, the enzymatic preparations were utilized as lyophilized cells.

A summary of the first set of results can be observed in Table 1 (for the whole set of data, see Table S1 in ESI). The substrate remained perfectly stable in the reaction medium (entry 1), and reacted with the different TAs tested: *R*-selective enzymes from *Arthrobacter* sp. (ArR-TA)[21] and its variant ArRmut11-TA,[20b] and

S-selective TAs from *Chromobacterium violaceum* (CV-TA)[22] and *Arthrobacter citreus* (ArS-TA),[23] affording β,β-difluoroamine **2a** in excellent conversions after 24 h, especially with ArR-TA and ArRmut11-TA (entries 2, 3, and 7). Except for the variant, the reactions proceeded at lower extent using a higher amine donor concentration (1 M) since these wild-type biocatalysts do not work appropriately in the presence of high 2-PrNH$_2$ loadings. Defluorinated carbonyl compounds, 2-fluoroacetophenone (**3a**) and acetophenone were detected in some cases just as traces (<3%). Satisfyingly, in most cases the enantiopure amine (>99%) was attained as corroborated by chiral GC (Table S2 in ESI).[24]

Apart from the chemoselectivity shown by TAs (amination *vs* hydrodefluorination), it was also noticeable the fact that excellent conversions were obtained with a small molar excess of the amine donor with regards to the ketone substrate (even just using a 2.5-fold excess, Table 1, entry 2), in contrast to the high excess that must be employed (at least 30-50 equiv.) in similar transformations with other carbonyl compounds.[25] This is obviously related to the thermodynamic equilibrium of the process. In this case, as we measured in a recent contribution,[16] the amination of an α,α-difluorinated ketone is highly favored (44 kJ mol^{-1}) with regards to the non-halogenated derivative. Similar effects have been discussed in the bioreduction of α-haloketones catalyzed by alcohol dehydrogenases,[26] or in the amination of α-oxygenated ketones with TAs.[27]

Table 2 Reactivity of α,α-difluoroketones **1a**-**j** with transaminases[a]

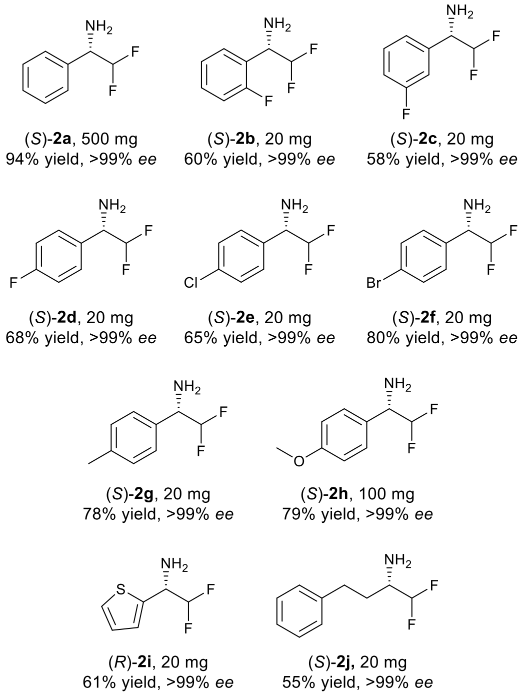


Entry	Substrate (R)	TA	**2a**-**j** (%)[b]	***ee* 2a**-**j** (%)[c]
1	**1a** (C$_6$H$_5$)	ArR	>99	>99 (*S*)[d]
2[e]	**1a** (C$_6$H$_5$)	ArR	>99	>99 (*S*)[d]
3	**1a** (C$_6$H$_5$)	CV	70	>99 (*R*)[d]
4	**1b** (*o*-F-C$_6$H$_4$)	ArRmut11	>99	>99 (*S*)[d]
5	**1c** (*m*-F-C$_6$H$_4$)	ArR	>99	>99 (*S*)[d]
6	**1c** (*m*-F-C$_6$H$_4$)	ATA-251	50	>99 (*R*)[d]
7	**1d** (*p*-F-C$_6$H$_4$)	ArR	>99	>99 (*S*)[d]
8	**1e** (*p*-Cl-C$_6$H$_4$)	ArR	>99	>99 (*S*)[d]
9	**1f** (*p*-Br-C$_6$H$_4$)	ArR	>99	>99 (*S*)[d]
10	**1g** (*p*-Me-C$_6$H$_4$)	ArR	>99	>99 (*S*)[d]
11	**1g** (*p*-Me-C$_6$H$_4$)	CV	53	>99 (*R*)[d]
12	**1h** (*p*-OMe-C$_6$H$_4$)	ArR	98	>99 (*S*)[d]
13	**1h** (*p*-OMe-C$_6$H$_4$)	CV	50	>99 (*R*)[d]
14	**1i** (2-thiophenyl)	ArR	>99	>99 (*R*)
15	**1j** (PhCH$_2$CH$_2$-)	ArR	>99	>99 (*S*)[d]
16	**1j** (PhCH$_2$CH$_2$-)	ArS	87	>99 (*R*)[d]

[a] For experimental details, see ESI. [b] Conversions measured by GC. [c] Enantiomeric excess values measured by chiral GC or HPLC. [d] Change in the CIP priority. [e] A deep eutectic solvent (2.5% v/v) composed by ChCl:glycerol 1:2 (mol:mol) was used as co-solvent instead of DMSO.

In order to study the scope of this transformation, various (hetero)aromatic α,α-difluoroketones (**1b**-**i**) were synthesized following a previously described methodology,[14] bearing substituents with different electronic properties at *ortho*, *meta* or *para* positions, also including a substrate with a thiophenyl ring (**1i**). Gratifyingly, in all examples tested under the previously optimized conditions (30 mM of the ketone and 100 mM of 2-PrNH$_2$), TAs exclusively formed the corresponding enantiopure β,β-difluoroamines **2b**-**i** (Tables 2 and S3 in ESI), not detecting the presence of the defluorinated ketones. Moreover, an aliphatic substrate was tested (**1j**), providing the enantiopure amine **2j** using two stereocomplementary TAs. Especially ArR-TA and its variant afforded high conversions into the *S*-amines.[24] Since CV-TA and ArS-TA did not produce the *R*-enantiomers at high extent, a commercially available kit of TAs was also tested (Tables S4-S9 in ESI), but unfortunately results were not improved. Due to the relevance that deep eutectic solvents have recently acquired as green media,[28] as a proof-of-concept, we replaced DMSO by a mixture of choline chloride (ChCl) and glycerol (1:2 mol:mol), reaching an excellent result in the biotransamination of ketone **1a** (entry 2).

Preparative bioaminations (20-100 mg) using ArR-TA or ArRmut11-TA were performed, employing acetonitrile as co-solvent instead of DMSO for an easier work-up, isolating the enantiopure products in good yields after an acid-base extraction procedure (55-82%, Fig. 2), and optical rotations were measured in order to assign the absolute configuration of the amines comparing their values with the ones described in the literature (Section 8 in ESI).[9,13] Also, a preparative transformation for the transamination of ketone **1a** (500 mg) using *E. coli*/ArR lyophilized cells in a 1:1 (w/w) loading was performed (Section 7.4 in ESI), obtaining enantiopure (*S*)-**2a** with 94% isolated yield. This result demonstrates the robustness of this enzymatic methodology to obtain enantiopure β,β-difluoroamines.


(*S*)-**2a**, 500 mg
94% yield, >99% *ee*

(*S*)-**2b**, 20 mg
60% yield, >99% *ee*

(*S*)-**2c**, 20 mg
58% yield, >99% *ee*

(*S*)-**2d**, 20 mg
68% yield, >99% *ee*

(*S*)-**2e**, 20 mg
65% yield, >99% *ee*

(*S*)-**2f**, 20 mg
80% yield, >99% *ee*

(*S*)-**2g**, 20 mg
78% yield, >99% *ee*

(*S*)-**2h**, 100 mg
79% yield, >99% *ee*

(*R*)-**2i**, 20 mg
61% yield, >99% *ee*

(*S*)-**2j**, 20 mg
55% yield, >99% *ee*

Fig. 2 β,β-Difluoroamines synthesized at preparative scale using heterologously expressed ArR-TA. Reaction scale, isolated yields and *ee* values are shown. For experimental details, see ESI.

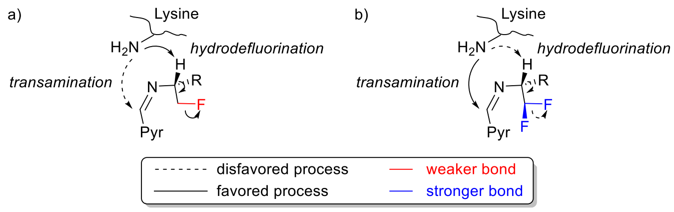


Fig. 3 Possible differences between the complex of PLP and: a) an α-fluoroketone, and b) an α,α-difluoroketone.

The fact that α,α-difluorinated ketones did not undergo hydrodefluorination in contrast to the monofluorinated analogs was an interesting finding. The dehalogenation process mediated by PLP-dependent biocatalysts such transaminases on α-fluoroketones and β-fluoroamines occurs in hand with the deactivation of the enzyme due to the formation of a covalent adduct of the substrate with PLP.[19,29] Since it was previously described that β,β-difluorinated amines could act as inhibitors of γ-aminobutyric acid aminotransferase (GABA-AT),[30] it was envisaged that these substrates would be preferentially defluorinated. However, this study shows that a wide set of TAs were able to aminate these derivatives, remaining the promiscuous hydrodefluorination as a minor and side activity. While the formation of the pyridoxamine 5′-phosphate complex with α-fluoroketones enlarges the C–F bond,[31] thus favoring the release of the halogen, it is also known that if the number of fluorine atoms linked to the same carbon increases, this enlarges the strength of the C–F linkages, and therefore, are less prone to be broken.[32] Moreover, since it is assumed that the catalytic lysine acts as the base to abstract the proton at α position of the imine and speed up the elimination of the F atom,[29a,33] the presence of a second fluorine atom can affect to the acidity of that proton and also can increase the steric hindrance at that position, abolishing the proton abstraction (Fig. 3). Studies to clarify this effect are currently being developed and will be reported in due course.

Conclusions

Chiral β,β-difluoroamines are highly valuable compounds due to their relevant biological activities. However, selective methods under mild reaction conditions to obtain these compounds have been scarcely reported. Herein we show that transaminases are able to catalyze the amination of (hetero)aromatic and aliphatic α,α-difluoroketones in a chemoselective way, since the possible promiscuous hydrodefluorination activity was hardly detected, thus affording the enantiopure β,β-difluoroamines. Moreover, due to the fact that the process is thermodynamically favored, a small molar excess of the amine donor was sufficient to give the desired products at high extent. The process could be easily scaled up to 500 mg. This contribution demonstrates once more that biocatalysts can be used as a valuable complement of other established synthetic methodologies, and that small changes in the substrate structure (*e.g.*, the number of F atoms at a carbon position), can largely influence the biocatalyst reactivity.

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Author Contributions

M.G.-R. has performed the experiments and analyzed the results. The study was conceptualized by I.L. Both authors contributed to the manuscript writing.

Conflicts of interest

There are no conflicts to declare.

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24 Notice that *R*-selective enzymes afforded the *S*-enantiomers and vice versa due to change in the CIP priority of the substituents (the small one has higher preference over the big one).

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