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COMMUNICATION

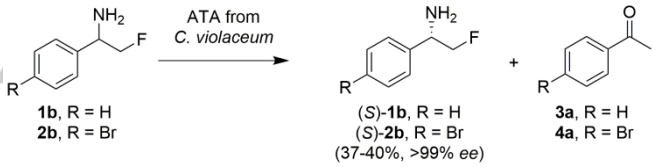
Asymmetric Synthesis of Primary and Secondary β-Fluoro-arylamines using Reductive Aminases from Fungi

Daniel González-Martínez,[a],[b] Aníbal Cuetos,[a] Mahima Sharma,[a] Marina García-Ramos,[b] Iván Lavandera,*[b] Vicente Gotor-Fernández*[b] and Gideon Grogan*[a]

Abstract: The synthesis of chiral amines is of central importance to pharmaceutical chemistry, and the inclusion of fluorine atoms in drug molecules can both increase potency and slow metabolism. Optically enriched β-fluoroamines can be obtained by the kinetic resolution of racemic amines using amine transaminases (ATAs), but yields are limited to 50%, and also secondary amines are not accessible. In order to overcome these limitations, we have applied NADPH-dependent reductive aminase enzymes (RedAms) from fungal species to the reductive amination of α-fluoroacetophenones with ammonia, methylamine and allylamine as donors, to yield β-fluoro primary or secondary amines with >90% conversion and between 85 and 99% *ee*. In addition, the effect of the progressive introduction of fluorine atoms to the α-position of the acetophenone substrate reveals the effect of mono-, di- and tri-fluorination on the proportion of amine and alcohol in product mixtures, shedding light on the promiscuous ability of imine reductase (IRED)-type dehydrogenases to reduce fluorinated acetophenones to alcohols.

Chiral amines are significant functional groups that feature prominently in many pharmaceuticals. There are many asymmetric methods for their synthesis that use transition metal catalysis coupled to chiral ligands,[1,2] but considerations of selectivity, sustainability and green chemistry have dictated that biocatalytic methods have achieved prominence in recent years.[3-5] The synthesis of fluoroamines represents a special case of chiral amine synthesis, as the addition of the fluorine atom can improve the efficiency of bioactive molecules through increased potency or slower metabolism.[6,7] β-Fluoroamines are very interesting derivatives as they can be excellent pyridoxal 5'-phosphate (PLP)-dependent enzyme inhibitors.[8] They can be synthesized simply through the ring opening of *N*-tosyl aziridines with TBAF,[9] but their asymmetric synthesis is rare, and complicated if chirality at both the amine and fluorine-bearing carbons is under consideration. In a recent example, Vara and Johnston used Brønsted base catalysts, such as $(MeO)_2$PBAM·HNTf, for the enantioselective synthesis of Boc-protected β-amino-α-fluoro-nitroalkanes from α-fluoro arylnitromethane and aldimine precursors.[10] Other examples using organocatalytic catalysts have been provided by Lindsley and co-workers,[11,12] who performed the enantioselective fluorination of *N*-sulfinyl aldimines, followed by nucleophilic addition using a Grignard reagent, providing the final compounds in high *dr* (>20:1).

More recently, we showed that amine transaminases (ATAs) can be applied to the 100 mg scale asymmetric synthesis of β-fluoroamines, with up to 99% *ee*., through the kinetic resolution of racemic substrates.[13] A series of racemic β-fluoro arylethylamines including **1b** and **2b** (**Scheme 1**) was converted by both (*S*)- and (*R*)- selective ATAs to give the corresponding enantiopure β-fluoroamines, in addition to acetophenones **3a** and **4a**, co-products that arise through the enantioselective tandem hydrodefluorination-deamination process that is catalyzed by the enzymes.



Scheme 1. Kinetic resolution of β-fluoroamines by a promiscuous hydrodefluorination-deamination process catalyzed by ATAs.[25]

However, the applicability of the method is compromised, owing to the maximum 50% yields that may be achieved, and the concomitant inhibition of the ATAs used, due to the formation of a covalent adduct with PLP in the active site of the enzyme.

A solution to the limited yield of the ATA-catalyzed process is presented by imine reductases (IREDs).[14,15] When presented with ketones, such as acetophenone, and a large excess of ammonia or other amine, these NADPH-dependent enzymes catalyze the asymmetric reduction of imines formed in solution, to give chiral amine products such as α-methylbenzylamine (**3b**) in a theoretically quantitative yield and with high enantiomeric excess (**Scheme 2**). A subgroup of IRED enzymes, reductive aminases (RedAms)[16-18] has also been shown to catalyze the formation of the iminium ion with certain ketone and amine partners, with the consequence that the coupling reaction is achieved with the supply of only an equimolar ratio of the substrates.[16,19] Interestingly, when presented with α,α,α-trifluoroacetophenone **5a** (**Scheme 2**), a promiscuous reduction of the ketone to the alcohol **5e** is performed by an IRED from *Streptosporangium roseum* (*Sr*IRED),[20] a reaction which has thus far not been observed in any other IRED/RedAm-catalyzed transformation of any carbonyl substrate.

These investigations prompted us to study the activity of RedAms as catalysts for the production of β-fluoro-arylamines such as **1b** from α-fluoroketones and amine precursors. We demonstrate that these enzymes are indeed effective catalysts for the production of optically enriched (*N*-substituted) β-fluoroamine products. We have also conducted a detailed analysis of the catalytic promiscuity of IRED-type dehydrogenases for the reduction of ketones fluorinated at the α-position, to the alcohol, in addition to amine, products.

[a] Dr Aníbal Cuetos, Dr Mahima Sharma, Prof. Gideon Grogan
Department of Chemistry
University of York
Heslington, York, YO10 5DD UK
e-mail: gideon.grogan@york.ac.uk
[b] Mr Daniel González-Martínez, Ms Marina García-Ramos, Prof. Iván Lavandera, Prof. Vicente Gotor-Fernández
Department of Organic and Inorganic Chemistry
University of Oviedo
Avenida Julián Clavería 8, 33006 Oviedo (Asturias), Spain
e-mail: lavanderaivan@uniovi.es; vicgotfer@uniovi.es
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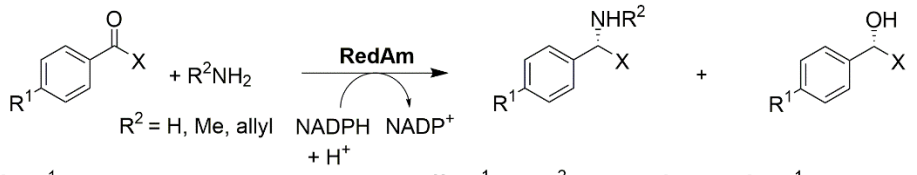

1a, R^1 = H, X = CH_2F
1b, R^1 = H, R^2 = H, X = CH_2F
1c, R^1 = H, R^2 = Me, X = CH_2F
1d, R^1 = H, R^2 = allyl, X = CH_2F
1e, R^1 = H, X = CH_2F

3a, R^1 = H, X = CH_3
3b, R^1 = H, R^2 = H, X = CH_3
3c, R^1 = H, R^2 = Me, X = CH_3
3d, R^1 = H, R^2 = allyl, X = CH_3
3e, R^1 = H, X = CH_3

5a, R^1 = H, X = CF_3
5e, R^1 = H, X = CF_3

6a, R^1 = H, X = CHF_2
6b, R^1 = H, R^2 = H, X = CHF_2
6c, R^1 = H, R^2 = Me, X = CHF_2
6d, R^1 = H, R^2 = allyl, X = CHF_2
6e, R^1 = H, X = CHF_2

7a, R^1 = F, X = CH_2F
7b, R^1 = F, R^2 = H, X = CH_2F
7e, R^1 = F, X = CH_2F

8a, R^1 = Cl, X = CH_2F
8b, R^1 = Cl, R^2 = H, X = CH_2F
8e, R^1 = Cl, X = CH_2F

2a, R^1 = Br, X = CH_2F
2b, R^1 = Br, R^2 = H, X = CH_2F
2e, R^1 = Br, X = CH_2F

9a, R^1 = I, X = CH_2F
9b, R^1 = I, R^2 = H, X = CH_2F
9e, R^1 = I, X = CH_2F

10a, R^1 = Me, X = CH_2F
10b, R^1 = Me, R^2 = H, X = CH_2F
10e, R^1 = Me, X = CH_2F

Scheme 2. RedAm-catalyzed transformations described in this study.

Biotransformations of fluorinated acetophenones by fungal RedAms

In initial experiments, α-fluoroacetophenone **1a** at 5 mM initial concentration, and on an analytical scale, was transformed using *Ad*RedAm from *Ajellomyces dermatitidis*[16] as the catalyst, in the presence of a large excess (250 mM) of methylamine ($MeNH_2$) and with glucose-6-phosphate (G6P) and glucose-6-phosphate dehydrogenase (G6PDH) to regenerate the NADPH cofactor. Under these conditions, we assume that the RedAms are acting in an IRED mode, binding and reducing the imine formed in solution, as acetophenones are not good substrates for equimolar reductive amination reactions.[16,19] Details on gene cloning and protein purification can be found in the Supporting Information (**SI**, **Sections III-IV**). The synthesis of substrates and product standards is detailed in **SI Section XI**. In initial experiments, conversions of up to 65% to the amine product **1c** were achieved, with only 3% evolution of alcohol product **1e** at 30 ºC (**Scheme 2**, **Table 1**, **entry 1**).

After optimization (**SI**, **Table S1**), 37 ºC was selected as the best temperature (**Table 1**, **entry 2**) and also an increase of the amine donor up to 500 mM (**Table 1**, **entry 3**) afforded a conversion of 92% into **1c**. Experiments (**Table 1**, **entries 4-9**) also showed that α-fluoroacetophenone (**1a**) was a superior substrate to acetophenone (**3a**) when aminated with either ammonia or methylamine, giving 18% and 46% conversion to products **1b** and **1c** respectively after 20 h, compared to 6% and 28% for the transformation of **3a** into **3b** or **3c**. However, in the case of the reaction of **1a** with ammonia, the fluorinated alcohol **1e** was detected in a significant amount of 14% (**Table 2**, **entry 4**). The proportion of amine to alcohol product was not only influenced by the amine donor, but also by the extent of fluorination on the acetophenone substrate (**Table 1**, **Figure 1**). In order to better compare the results, enzymatic transformations were stopped at 20 h. Hence acetophenone (**3a**) was transformed only to amine products when incubated with *Ad*RedAm and ammonia, methylamine or allylamine; α-fluoroacetophenone (**1a**) was converted into a mixture of products only when ammonia was the donor; while α,α-difluoroacetophenone (**6a**) was transformed to predominantly the alcohol when ammonia was the donor, but to more equal proportions of alcohol and amine with methylamine or allylamine; finally, α,α,α-trifluoroacetophenone (**5a**) was transformed exclusively into the alcohol **5e** in each case.

Table 1. Transformations of α-fluoroacetophenone (**1a**, X=CH_2F) with *Ad*RedAm and several amine donors. Reactions between acetophenone (**3a**, X=CH_3) and amine donors have been added for comparison.

Entry	Ketone	Amine Donor	t (h)	Amine b-d (%)[a]	Alcohol e (%)[a]
1	**1a**[b]	$MeNH_2$	64	65	3
2	**1a**	$MeNH_2$		77	4
3	**1a**	$MeNH_2$[c]		92	5
4	**1a**	NH_3	20	18	14
5		$MeNH_2$		46	<1
6		$AllylNH_2$		27	<1
7	**3a**	NH_3	20	6	<1
8		$MeNH_2$		28	<1
9		$AllylNH_2$		35	<1

[a] Conversions were determined by GC-FID analysis of the reaction crude. Reaction conditions: ketone (5 mM), amine (50 eq.), *Ad*RedAm (1 mg/mL), $NADP^+$ (1 mM), G6P (30 mM), G6PDH (2 U), MeCN (2% v/v), buffer Tris·HCl (100 mM, pH 9.0, 500 µL) at 37 ºC. [b] Reaction was carried out at 30 ºC. [c] 100 eq. of amine donor were used.

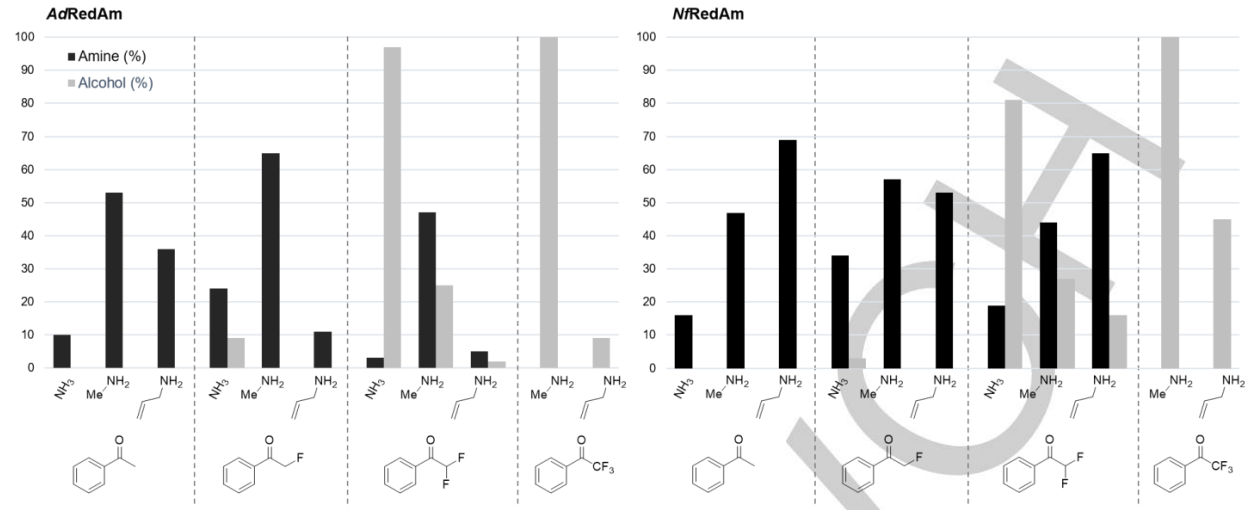

Figure 1. Percentages of amine (black) and alcohol (grey) products obtained by biotransformation (t = 20 h) of fluorinated acetophenone derivatives by *Ad*RedAm (left) and *Nf*RedAm (right). These results are tabulated in **Table S2** in the Supporting Information.

A similar trend was observed for equivalent biotransformations catalyzed by *Nf*RedAm from *Neosartorya fumigatus* (**Figure 1**). Additional results obtained for *At*RedAm from *Aspergillus terreus*,[19] *Nfis*RedAm from *Neosartorya fischeri*, and *Asp*RedAm from *Aspergillus oryzae*[16] can be found in the Supporting Information (**SI**, **Section VII**, **Figure S5**, **Table S2**). It is important to note that in control reactions, which were performed in the absence of RedAm, but with both the cofactor and the cofactor recycling system, neither amination reactions, nor reduction of the ketones to alcohols, were observed. The stereoselectivity of the RedAm-catalyzed biotransformations is shown in **Table 2** and in the **SI** (**Table S3**), and the analytical methods and results detailed in the Supporting Information (**SI**, **Section X**), with details on the assignment of absolute configuration (**SI**, **Section XII**). In most cases the enantioselectivity of the reactions was very high (up to 99%), and although these enzymes have been described as (*R*)-selective,[16,19] products were of the (*S*)-enantiomeric series, except amine **3b**, due to the change in Cahn-Ingold-Prelog (CIP) priority. The *ee* values for amines **1b**, **6c** and **6d** were especially high, with products obtained in enantiopure form for most examples (**SI**, **Table S3**).

*Ad*RedAm and *Nf*RedAm appeared to be the best biocatalysts overall for the transformations (**Table 2**), giving amine products with high conversions and in excellent *ee* using an excess of the amine donor (80-100 eq.) and in the presence of a small percentage of an organic co-solvent. Alcohols that were formed in the reactions were also of the (*S*)-enantiomeric series and displayed *ee* values >97% in many cases (**Table S3**). RedAms also catalyze the amination of *para*-halogenated α-fluoroacetophenones with F (**7a**), Cl (**8a**), Br (**2a**) and I (**9a**) as substituents with ammonia (**SI**, **Section IX**). Although activities decreased with increasing size of the halogen atom, enantioselectivity was largely conserved, obtaining (*S*)-amine products with high to excellent *ee* (**Table 2**). With these substrates, in no case were the corresponding alcohol derivatives observed The *p*-iodo derivative **9a** was only poorly transformed using ammonia, as was the *p*-methyl derivative **10a** (<2% conversion). 2-chloro α-fluoroacetophenone (not shown) was also transformed by *Ad*RedAm with only poor conversion (<5%) using either ammonia or methylamine.

The biotransformation of **1a** to **1c** by *Ad*RedAm was performed on a 70 mg scale, giving a GC conversion of 68% and a crude yield of 55% following extractive work up as described in **SI Section XIII**). Column chromatography yield the pure product in 44% yield with 96 % *ee*..

Table 2. Conversions and *ee* in the reductive amination of fluorinated acetophenones using RedAms and different amine donors (**b**: NH_3, **c**: $MeNH_2$, **d**: $AllylNH_2$).

Product	Enzyme	eq. amine	Co-solvent	t (h)	c (%)[a]	*ee* (%)[a]
1b[b]	*Ad*RedAm	80	MeOH (1%)	44	81	99
1c	*Ad*RedAm	100	MeOH (1%)	20	84	96
1d	*Nf*RedAm	100	MeOH (1%)	20	69	93
6c[c]	*Nf*RedAm	100	MeCN (2%)	70	71	99
6d	*Nf*RedAm	100	MeCN (2%)	20	65	99
7b	*Nfis*RedAm	100	MeOH (1%)	40	76	99
8b	*Nf*RedAm	100	MeOH (1%)	40	76	99
2b	*Nf*RedAm	100	MeOH (1%)	40	34	85

[a] Conversions and enantiomeric excess values were determined by GC-FID analysis of the reaction crude after *in situ* derivatisation with acetic anhydride. [b] 10 mM **1a**. [c] 0.05 mM $NADP^+$ used to minimise the alcohol **6e** formation (16%).

Studies on the promiscuous reduction of ketones fluorinated at the α-position by IREDs/RedAms

In addition to expanding the applicability of RedAms to the production of primary and secondary β-fluoro-arylamines, this study also sheds more light on the observation that α,α,α-trifluoroacetophenone **5a** is reduced exclusively to the alcohol by a promiscuous reaction catalyzed by the *Sr*IRED from *Streptosporangium roseum*.[20] We observed this to be a general trend for other IRED/RedAm enzymes, particularly for **5a**, but also to a lesser extent when the mono- and di-fluorinated substrate analogues **1a** and **6a** are used. A number of factors may contribute to the promiscuous activity observed. The results suggest that the hydride transfer process is easier for fluoroacetophenones than for non-fluorinated analogs, as the electrophilicity of the carbonyl carbon is steadily increased with successive fluorine substitutions. When comparing the Gibbs free energies of the carbonyl reduction using DFT calculations, we observed that reduction of **1a**, **6a** and **5a** were 20.2, 37.0 and 40.1 kJ mol^{-1} more favorable, respectively, than for the reduction of **3a** (**SI Section XIV**, **Table S11**). On the other hand, the imine formation from **1a**, **6a** and **5a** was also more favorable with respect to acetophenone (**SI**, **Tables S11-S12**), but in this case the influence of the number of fluorine atoms was not significant (15.8-17.9 kJ mol^{-1}). Overall, these energetic values are in agreement with the formation of the alcohols, especially for di- and tri-fluorinated ketones, when compared to non- and also mono-fluorinated ketones. However, the different amine:alcohol product ratios obtained when using different amine donors for the same ketone substrate, such as **1a** and **6a** suggests that molecular recognition of both substrates may also play a part in switching the chemoselectivity of the reaction. For the reactions presented, where a large excess of amine is used, a preference for alcohol over amine production (or *vice versa*) must reflect the preferred binding of the ketone substrate over the imine recruited from solution within the active site. In a recent study by one of our groups together with Nestl and co-workers, we determined the structure of the imine reductase *Sr*IRED in complex with the hydrate of α,α,α-trifluoroacetophenone.[21] This revealed that, in the presence of this fluorinated substrate, two of the α-fluorine atoms make a direct interaction with one of the ribose hydroxyl groups of NADP$^+$, and may, in the case of the fluoroketone substrate **5a**, draw the electrophilic carbon of the substrate within a suitable distance for reduction by the cofactor. These interactions may be less pronounced in either α,α-difluoroacetophenone **6a** or α-fluoroacetophenone **1a** and of course absent for acetophenone **3a**, in which series a decreasing proportion of alcohol products are observed. The increasing proportion of amine products obtained when **1a** or **6a** are aminated with ammonia, methylamine and allylamine is therefore a reflection of the increasingly successful competition of the *N*-alkylated imine for the active site over the fluoroketone. These phenomena therefore constitute an interesting example of substrate-directed enzyme promiscuity dependent upon both electronic and thermodynamic effects.

The synthesis of chiral amines from readily available prochiral substrates remains a challenge for asymmetric catalysis, and IRED/RedAm-type enzymes can offer sustainable and atom-efficient solutions to some of these problems. The recognition that α-fluorinated ketones can serve as substrates for alkyl-amination by RedAms, as well as for reduction to chiral alcohols, introduces a new reaction to the available biocatalytic toolbox, and provides a platform for improvement of these processes through both enzyme and reaction engineering.

Experimental Section

Details of gene cloning and expression, enzyme purification and assay, synthesis of substrates and product standards, GC and HPLC analyses, biotransformation protocols and modelling/thermodynamics studies can be found in the Supporting Information.

Acknowledgements

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Keywords: oxidoreductase • imine reductase • reductive amination • fluoroamine • biocatalysis

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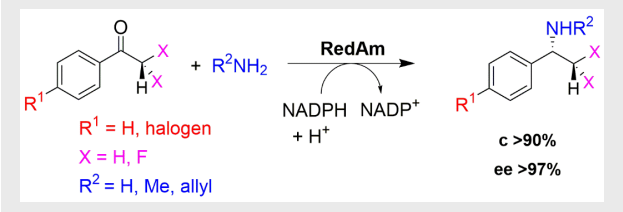

A series of enantioenriched primary and secondary β-fluoroamines can be obtained through a stereoselective reductive amination process catalyzed by different reductive aminases with a selection of amine donors.

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