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Chemo- and Stereoselective Synthesis of Fluorinated Amino Alcohols through One-pot Reactions using Alcohol Dehydrogenases and Amine Transaminases

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Abstract. A series of amino alcohols have been prepared in a chemo-, diastereo- and enantioselective fashion starting from the corresponding (het)aryl diketones, avoiding tedious chemical protection and deprotection steps. Different alcohol dehydrogenases have been able to selectively reduce the more reactive trifluoroacetyl groups under optimized conditions, while amine transaminases catalyzed the biotransamination of the less hindered acetyl groups. Based on the different reactivity of the acetyl and trifluoroacetyl groups, the design of sequential and concurrent cascades was investigated. The proper selection of the enzymes permits the synthesis of amino alcohol stereoisomers in high to excellent yields (86->99% conversion) and remarkable stereocontrol (up to >99% *de* and >99% *ee*) using an aqueous medium and mild reaction conditions.

Keywords: Asymmetric synthesis; Biocatalysis; Bioreduction; Biotransamination; Chiral amino alcohols

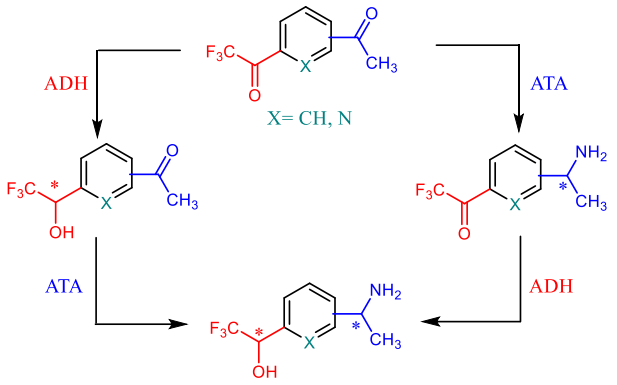
Introduction

Amino alcohols attract growing interest in medicinal and organic chemistry.[1] Their importance is highlighted in asymmetric catalysis when considering the use of enantiomerically pure forms as chiral auxiliaries in very diverse chemical transformations.[2] The use of enzymes in organic synthesis has shown great potential for the stereoselective preparation of amino alcohols, a variety of biocatalyst classes emerging as suitable tools for this aim.[3] The use of aldolases results particularly attractive due to the high stereoselectivity displayed in the formation of C–C bonds,[4] although their nucleophile specificity ballasts their use towards a broad amino alcohol panel.

The development of one-pot chemoenzymatic and multienzymatic transformations offers interesting advantages over classical stepwise transformations, avoiding the isolation and purification of reaction intermediates and allowing to increase molecular complexity in a straightforward manner.[5] Interestingly, these multi-step one-pot processes can combine the productivity of chemical catalysis (i.e. metal catalysis, organocatalysis…) with the stereoselectivity displayed by enzymes, making also feasible the use of several biocatalysts to achieve complex transformations.[6] Amino alcohol asymmetric multienzymatic syntheses have not gone unnoticed, and selected combinations of enzymes have been successfully developed in recent years. Thus, bienzymatic synthesis combining transketolases,[7] pyruvate aldolases,[8] pyruvate decarboxylases[9] or alcohol dehydrogenases (ADHs)[10] with amine transaminases (ATAs) have provided efficient and selective access to amino alcohol diastereoisomers, and even the possibility of using three enzymes (epoxide hydrolases, ADHs and ATAs) in a linear cascade set-up has been successfully reported.[11]

The synthesis of various families of fluorine-containing compounds has attracted great attention in recent decades[12] due to the importance of organofluorinated compounds in medicinal chemistry[13] and asymmetric catalysis.[14] For that reason, we decided to explore the potential of biocatalysis for the production of chiral fluorinated amino alcohols, attempting one-pot bioreduction and biotransamination reactions (Scheme 1).

ADHs[15] and ATAs[16] are highly versatile biocatalyst types, able to transform carbonyl groups into the corresponding alcohols and amines, respectively. Therefore, after synthesizing a series of representative (het)aryl diketones, the behaviour of different made in house and commercial enzymes has been studied, searching for the development of chemo- and stereoselective methods to modify diketones bearing methyl and trifluoromethyl groups.



Scheme 1. Development of bienzymatic transformations for the production of fluorinated amino alcohols starting from (het)aryl diketones.

The compatibility of both types of biocatalysts has been investigated, trying to set up one-pot strategies towards optically active amino alcohols through both two-step sequential and concurrent approaches.[5a] Whenever possible and trying to present a simple experiment set-up, attempts will be made to develop a concurrent process where all the enzymes and reagents are added at the beginning and the two reactions take place simultaneously. However, the development of two-step sequential strategies will be also explored especially when enzyme inhibition effects can produce a decrease in either the yield or the selectivity of the global process.

Results and Discussion

For clarity, the chemical structures of diketones **1a-f**, hydroxy acetophenones **2a-f**, hydroxy di- or trifluoroacetophenones **3a-f**, diols **4a-f**, amino di- or trifluoroacetophenones **5a-f**, amino acetophenones **6a-f**, diamines **7a-f** and amino alcohols **8a-f** are depicted in the Page S2 of the Electronic Supporting Information.

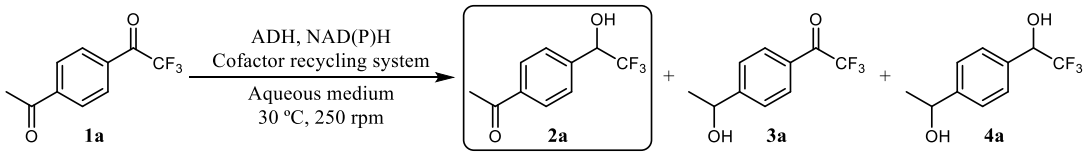
Enzyme-catalyzed reduction and transamination of trifluoroacetyl acetophenones

Previous metal-catalyzed enantioselective reduction studies of diketone **1a** allowed the chemoselective formation of the corresponding hydroxy ketone (*S*)-**2a** through asymmetric hydrogenation of the trifluoroacetophenone moiety using chloride-bridged dinuclear rhodium(III) complexes bearing Josiphos-type diphosphine ligands,[17] while its antipode (*R*)-**2a** was synthesized by using diethylzinc as the β-hydrogen donor and a phosphinamide chiral ligand.[18]

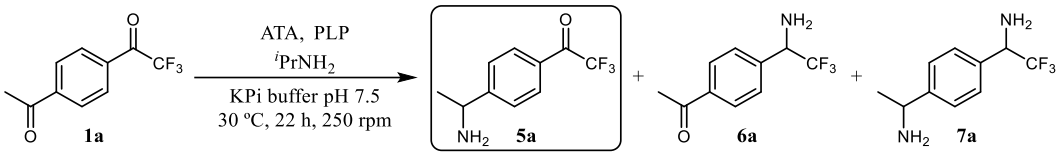
Inspired by the exquisite reactivity display by ADHs towards the bioreduction of prochiral methyl/trifluoromethyl diketones,[19] the synthesis of *para*-, *meta*- and *ortho*-substituted aryl diketones **1a-c** possessing the acetyl and trifluoroacetyl groups was undertaken, starting from the corresponding 2'-, 3'- or 4'-bromoacetophenones (see the Supplementary Information).[19,20] Next, the bioreduction of **1a** was studied, illustrating the three possible products in the Scheme 2: the expected hydroxy ketone **2a** by reduction of the trifluoroacetyl group; the alcohol **3a** in case that the reduction of the methyl ketone would be preferred; and the diol **4a** after modification of both carbonyl groups.

Two types of ADHs were employed: An own collection of overexpressed enzymes that includes the ones from *Ralstonia* species (*Ras*ADH),[21] *Sphingobium yanoikuyae* (*Sy*ADH),[22] *Thermoanaerobacter* species (ADH-T),[23] *Lactobacillus brevis* (*Lb*ADH),[24] *Thermoanaerobacter ethanolicus* (*Te*SADH)[25] and *Rhodococcus ruber* (ADH-A);[26] Commercial enzymes such as the evo-1.1.200 from Evoxx Technologies[27] and a kit from Codexis Inc.

A previous study dealing with the stereoselective bioreduction of methyl/trifluoromethyl diketones **1a** and **1b** showed that most of the tested ADHs led to mixtures of products.[19] Interestingly, Grau and co-workers reported the chemoselective reduction of the corresponding trifluoroacetyl group as the favoured reaction. In our case, we decided to firstly test the ADH from *Ralstonia* species (*Ras*ADH) overexpressed in *E. coli*, observing a complete conversion of diketone **1a** into the diol **4a** under standard conditions (Tris·HCl buffer pH 7.5, glucose and glucose dehydrogenase for cofactor recycling, 30 ºC and 6 h at 250 rpm). Assuming that due to the greater reduction potential of the trifluoromethyl ketone the reaction proceeds through the formation of hydroxy ketone **2a** intermediate, the possibility to stop in the monoreduction stage was attempted by using a single equivalent of glucose instead of the 3 equiv. employed in the first test. This would allow to generate only one equivalent of the real reducing agent, the NADPH cofactor. Gratifyingly, monoalcohol (*R*)-**2a** was exclusively obtained with a 97% *ee*, assigning the absolute configuration of the alcohol based on the known selectivity of the enzyme.



Scheme 2. ADH-catalyzed reduction of **1a**.



Scheme 3. ATA-catalyzed transamination of **1a**.

Similarly, commercial evo-1.1.200 led to enantiopure (*S*)-**2a** in quantitative conversion either using the enzyme-coupled approach (glucose/GDH) or the substrate-coupled strategy with 1 equiv. of isopropanol (^{i}PrOH). Interestingly, optimization of the reaction conditions in terms of nature and percentage of co-solvent led to excellent selectivities and complete conversions towards (*S*)- and (*R*)-**2a** when using 2.5% of 1,4-dioxane (Table S1), obtaining 97% isolated yield for the *Ras*ADH in a semi-preparative reaction (21.6 mg of substrate). Extensive screening with a new kit of Codexis Inc. ADHs allowed the identification of multiple ketoreductases (KREDs also called ADHs, Table S2), providing access to enantiopure hydroxy ketone **2a** enantiomers.

Moving to the bioreduction of the *meta*-substituted diketone **1b**, the *Ras*ADH quantitatively led to the diol **4b** using an excess of the glucose for cofactor recycling purposes. The chemoselective reduction of the trifluoroacetyl group was possible when using just one equivalent of glucose, although in this case the hydroxy ketone (*R*)-**2b** was isolated with a modest selectivity (40% *ee*). Later, other *E. coli* overexpressed ADHs were tested (ADHs from *Rhodococcus ruber*, *Sphingobium yanoikuyae* and *Thermoanaerobacter* species), although the best results towards (*R*)-**2b** were found with the commercial KRED-NADH-101 (>99% conversion, 98% *ee*). Complementary, five commercial ADHs, including the evo-1.1.200, led to quantitative conversions towards enantiopure (*S*)-**2b** (Table S3).

Finally, the diketone **1c** with the carbonyl groups in relative *ortho* position forms a highly stable cyclic double hemiacetal in aqueous medium (Scheme S4), which did not show any reactivity when testing a series of ADHs and ATAs.

At this point, the study of the biotransamination of diketones **1a** and **1b** was undertaken using: (i) four lyophilized whole cell biocatalysts overexpressed in *E. coli*, including the (*S*)-selective ATAs from *Chromobacterium violaceum* (*Cv*TA)[28] and *Arthrobacter citreus* (*Ar*STA),[29] and the (*R*)-selective wild-type transaminase from *Arthrobacter* sp. (*ArR*TA)[30] and its engineered variant *ArRmut11*;[31] (ii) a commercial ATA kit from Codexis Inc. In all cases, the diketone substrate was used in 20 mM concentration and isopropylamine (^{i}PrNH$_2$) was used in a large excess (1.0 M, 50 eq.) to shift the reaction equilibrium towards amine synthesis.[32]

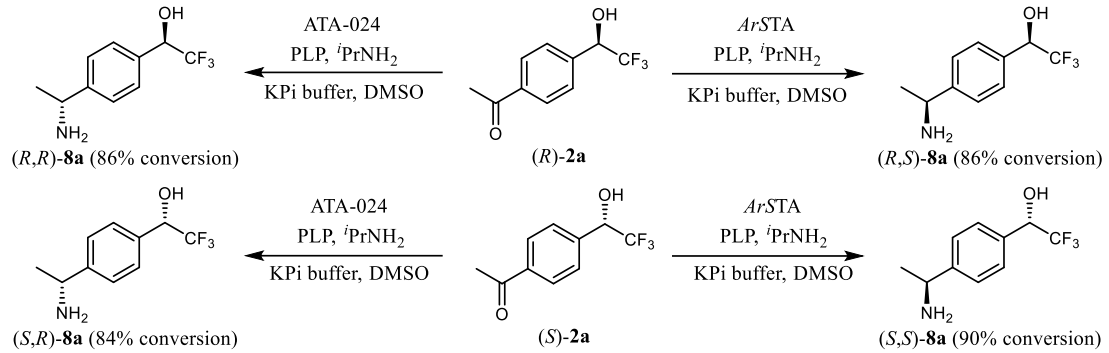
The biotransamination of diketone **1a** was attempted under the reaction conditions previously optimized in our research group (Scheme 3).[33] Most of the ATAs selectively modified the less bulky methyl ketone, leading to the amino ketone **5a** in up to 85% conversion, observing excellent selectivities in all cases (98->99%, Tables S4-S7). The best results were obtained with the *Ar*STA and ATA-238, obtaining the enantiopure (*S*)-**5a** in 80% and 85% conversion, respectively, while the ATA-412 led to the (*R*)-**5a** with a 76% conversion. Experiments were also performed in an open system to favour the shifting of the reaction equilibrium towards amine synthesis, increasing from 77 to 94% at 40 ºC after 24 h and, reaching quantitative conversion after 48 h at 30 ºC (Table S6).

Next, the biotransamination of 1-(3-acetylphenyl)-2,2,2-trifluoroethan-1-one (**1b**) was considered, achieving the pursued modification of the acetyl group towards the enantiopure (*S*)-**5b** in a 73% conversion with the *Ar*STA. Complementary, ATA-303 was found as the best (*R*)-selective ATA, obtaining the enantiopure (*R*)-**5b** in 68% yield using 1,4-dioxane as co-solvent and doubling the amount of enzyme (Table S8).

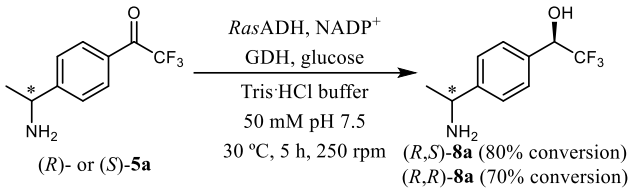
Towards the cascade bienzymatic synthesis of all possible stereoisomers of amino alcohols 8a and 8b

Once that chemo- and stereoselective modifications were successfully developed, the synthesis of the four possible stereoisomers of the amino alcohol **8a** was achieved starting from the hydroxy acetophenone **2a** (Scheme 4, 84-90% conversion). Alternatively, when starting from the amino trifluroethanone **5a** only the (*R,S*)- and (*R,R*)-**8a** diastereoisomers were obtained using the *Ras*ADH-catalyzed bioreduction (Scheme 5, 70-80% conversion), while the synthesis of the other two isomers was not possible since the evo-1.1.200 ADH did not display any activity over **5a**.

Based on the excellent selectivity displayed by selected ADHs and ATAs, next efforts were focused in the development of one-pot sequential or concurrent approaches (Table 1 and Table 2). Herein, a key issue is the use of a reaction medium compatible with both enzyme classes, which would avoid changes of buffer composition and pH along the process. To achieve this, a phosphate buffer containing ^{i}PrNH$_2$ (1.0 M) was employed. First, the sequential approach was conducted by developing the bioreduction of **1a** and, once completed the corresponding ATA and the PLP were added to perform the biotransamination step. However, the presence of ^{i}PrNH$_2$ provokes a negative effect in the ADH activity, effect that was alleviated using longer reaction times (16 h instead of 3 h) and doubling the ADH loading (Table S9), to obtain both hydroxy ketone **2a** enantiomers in quantitative conversions.



Scheme 4. Synthesis of all four **8a** stereoisomers by biotransamination of the hydroxy ketone **2a** enantiomers (2,2 mg, 20 mM). Reaction conditions: *Ar*STA (10.0 mg) or ATA-024 (2.0 mg), PLP (1 mM), $^{i}PrNH_2$ (1.0 M), DMSO (2.5% v/v) in KPi buffer (500 µL, 100 mM, pH 7.5) at 30 ºC and 250 rpm for 22 h.

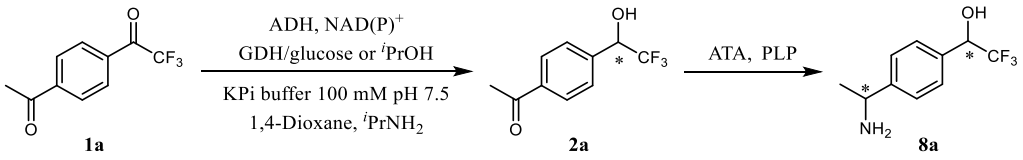


Scheme 5. *Ras*ADH-catalyzed bioreduction of **5a** enantiomers (2.2 mg, 20 mM) towards (*R*,*S*)- and (*R*,*R*)-**8a** diastereoisomers.

The best results have been summarized in Table 1, finding good activities for the production of all four stereoisomers in 68-79% conversion, highlighting the excellent diastereo- and enantioselectivities attained in all cases (>96% *de* and >99% *ee*). Interestingly, higher conversions were achieved by performing the second step (biotransamination) open to air that favours the *in situ* acetone removal, reaching the four diastereoisomers in 90-96% conversion (Table 2).

Next, the development of a concurrent cascade was investigated (Table S10), displaying the best results in Table 2 (95->99% conversion with complete stereoselection). Appropriate conditions were found by halving the concentrations of the substrate (from 20 mM to 10 mM of **1a**) and all the cofactors and reagents required in both reactions. At the same time, an increase in the ADH units favoured the reduction over the transamination process, while the use of an open-air system allowed a significant improvement in the conversion into the corresponding amino alcohol **8a**.

Table 1. Sequential one-pot synthesis of amino alcohol **8a** using ADHs and ATAs.[a]



Entry	ADH	ATA	Amino alcohol **8a**	*c* (%)[b]	*de* (%)[c]	*ee* (%)[c]
1	*Ras*ADH	*Ar*STA	(*R*,*S*)	79	98[d]	>99
2	*Ras*ADH	TA-P1-G05	(*R*,*S*)	68	98[d]	>99
3	*Ras*ADH	ATA-024	(*R*,*R*)	78	98[e]	>99
4	*Ras*ADH	ATA-025	(*R*,*R*)	77	98[e]	>99
5	*Ras*ADH	ATA-033	(*R*,*R*)	75	98[e]	>99
6	evo-1.1.200	*Ar*STA	(*S*,*S*)	64	97[f]	>99
7	evo-1.1.200	TA-P1-A06	(*S*,*S*)	71	97[f]	>99
8	evo-1.1.200	ATA-024	(*S*,*R*)	68	>99	>99
9	evo-1.1.200	TA-P2-B01	(*S*,*R*)	65	>99	>99

[a] Reaction conditions: **1a** (2.2 mg, 0.01 mmol), 1,4-dioxane (12.5 µL, 2.5% v/v), ADH (2.0 mg *Ras*ADH or 0.2 mg evo-1.1.200), glucose (20 mM), GDH (0.1 mg) in phosphate buffer (100 mM, pH 7.5, 500 µL total volume) containing isopropylamine (1.0 M) at 30 ºC and 250 rpm for 16 h; next the corresponding ATA (10.0 mg *Ar*STA or 4.0 mg commercial ATAs) and PLP (1 mM) at 30 ºC and 250 rpm for additional 20 h.

[b] Conversion values into product **8a** were measured by GC analysis of the reaction crude.

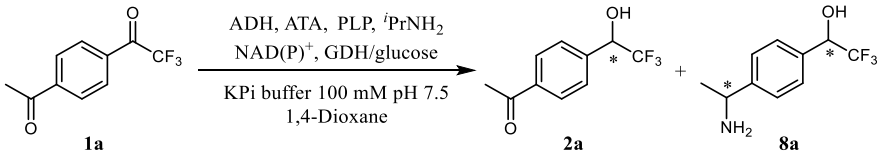
[c] Diastereomeric and enantiomeric excess values measured by HPLC after derivatization of the amino alcohol **8a** with Ac_2O and DMAP, and quick filtration through silica gel; n.m: not measured.

[d] 1% of the (*S*,*S*)-diastereoisomer was observed.

[e] 1% of the (*S*,*R*)-diastereoisomer was observed.

[f] 1.5% of the (*S*,*R*)-diastereoisomer was observed.

Table 2. Sequential and concurrent one-pot synthesis of amino alcohol **8a** under optimized conditions.



			Sequential[a]				Concurrent[b]			
Entry	ADH	ATA	**2a** (%)[c]	**8a** (%)[c]	*de* (%)[d]	*ee* (%)[d]	**2a** (%)[c]	**8a** (%)[c]	*de* (%)[d]	*ee* (%)[d]
1	*Ras*ADH	*ArS*TA	7	93 (*R*,*S*)	98	>99	5	95 (*R*,*S*)	98	>99
2	evo-1.1.200	*ArS*TA	10	90 (*S*,*S*)	97	>99	3	97 (*S*,*S*)	97	>99
3	*Ras*ADH	ATA-024	4	96 (*R*,*R*)	98	>99	2	98 (*R*,*R*)	98	>99
4	evo-1.1.200	ATA-024	4	96 (*S*,*R*)	>99	>99	<1	>99 (*S*,*R*)	>99	>99

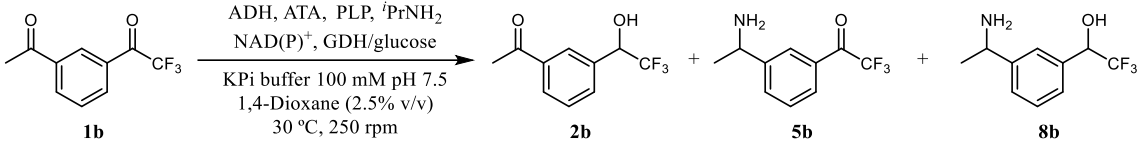
[a] Sequential mode: **1a** (4.4 mg, 0.02 mmol, 20 mM), ADH (6.0 mg *Ras*ADH or 0.2 mg evo-1.1.200), NAD(P)$^+$ (0.5 mM), GDH (0.1 mg), glucose (20 mM), 1,4-dioxane (25 µL, 2.5% v/v) in phosphate buffer (100 mM, pH 7.5, 1.0 mL total volume) containing isopropylamine (1.0 M) at 250 rpm (4 h with *Ras*ADH or 24 h with evo-1.1.200; second step: ATA (20 mg *ArS*TA or 10.0 mg ATA-024) and PLP (1 mM) during 24 h at 250 rpm and 30 ºC in an open to air system.

[b] Concurrent mode: **1a** (2.2 mg, 0.01 mmol, 10 mM), ATA (10.0 mg *ArS*TA or 5.0 mg ATA-024), ADH (10.0 mg *Ras*ADH or 1.0 mg evo-1.1.200), PLP (0.5 mM), NAD(P)$^+$ (0.25 mM), GDH (0.05 mg), glucose (10 mM), isopropylamine (0.5 M), 1,4-dioxane (25 µL, 2.5% v/v) in phosphate buffer (100 mM, pH 7.5, 1.0 mL total volume) at 250 rpm and 30 ºC during 48 h in an open to air system.

[c] Percentage of products was measured by GC analysis of the reaction crude.

[d] Diastereomeric and enantiomeric excess values measured by HPLC after derivatization of the amino alcohol **8a** with Ac_2O and DMAP, and quick filtration through silica gel; n.m: not measured.

Table 3. Sequential and concurrent cascade one-pot synthesis of amino alcohol **8b** under previously optimized conditions.



Entry	Approach	ADH	ATA	**1b** (%)[a]	**2b** (%)[a]	**5b** (%)[a]	**8b** (%)[a,b]
1	Sequential	KRED-NADH-101	*ArS*TA	<1	8	<1	92 (*R*,*S*)
2		evo-1.1.200	*ArS*TA	<1	5	<1	95 (*S*,*S*)
3		KRED-NADH-101	ATA-251	<1	<1	<1	>99 (*R*,*S*)
4		evo-1.1.200	ATA-251	<1	3	<1	97 (*S*,*S*)
5		KRED-NADH-101	ATA-303	<1	5	17	78 (*R*,*R*)
6		evo-1.1.200	ATA-303	<1	8	<1	92 (*S*,*R*)
7	Concurrent	KRED-NADH-101	*ArS*TA	<1	20	<1	80 (*R*,*S*)
8		evo-1.1.200	*ArS*TA	5	20	13	62 (*S*,*S*)
9		KRED-NADH-101	ATA-303	<1	10	<1	90 (*R*,*R*)
10		evo-1.1.200	ATA-303	<1	4	<1	96 (*S*,*R*)

[a] Percentage of products was measured by GC analysis of the reaction crude.

[b] Absolute configurations of the major stereoisomers appear in parentheses (all of them found to be enantio- and diastereomerically pure by HPLC analysis).

At this point, the extension of this methodology was explored, so first, the amino alcohol **8b** was synthesized starting from the diketone **1b**. The more active and selective enzymes found for the production of **8a** diastereomers were employed: (i) evo-1.1.200 and KRED-NADH-101 as ADHs (Table S3); (ii) *ArS*TA, ATA-251 and ATA-303 as ATAs (Table S8), reaching all four possible stereoisomers in enantio- and diastereomerically pure form (Table 3). In this case, the sequential approach resulted to be the best strategy, since the hydroxy ketone **2b** and the amino ketone **5b** intermediates were observed as subproducts in the concurrent reactions (4-33%).

Moving from trifluoromethyl/methyl diketones to a difluoroacetyl/acetyl substrate

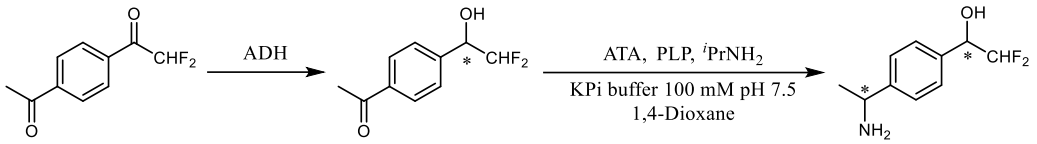
Next, the presence of a difluoroacetyl group instead of a trifluoracetyl one was considered in order to have a deeper knowledge of the ADH and ATA reactivity. For that reason, the *para*-substituted difluoroacetophenone **1d** was synthesized and subjected to bioreduction and biotransamination experiments. Regarding the bioreduction of diketone **1d**, (*S*)- and (*R*)-**2d** were satisfyingly obtained using complementary (*R*)-selective *Ras*ADH (98% *ee*) and (*S*)-selective evo-1.1.200 (>99% *ee*). However, the biotransamination reactions usually led to mixtures of

the amino ketones **5d** and **6d** and the diamine **7d** with some of the commercial enzymes (Table S12), while a very low reactivity was found with other ATAs (*Cv*TA or *ArS*TA, <3%).

Focusing on the preparation of the amino alcohol **8d** stereoisomers, the hydroxy ketones (*R*)- and (*S*)-**2d** were subjected to biotransamination reactions, finding active ATAs for the production of all four stereoisomers with conversions between 50 and 81% and complete selectivity (Table 4).

Gratifyingly, under optimized conditions, both sequential and concurrent cascades led to the complete consumption of the diketone **1d** with excellent stereoselectivity (Table 5). Therefore, the four stereoisomers of **8d** were obtained in quantitative conversion through the concurrent approach (entries 5-8), while some hydroxy ketone **2d** was observed using the sequential approach (entries 1-4).

Table 4. Stereoselective bioreduction of **1d** followed by biotransamination of the resulting hydroxy ketone **2d** (data corresponds only to the biotransamination process).[a]



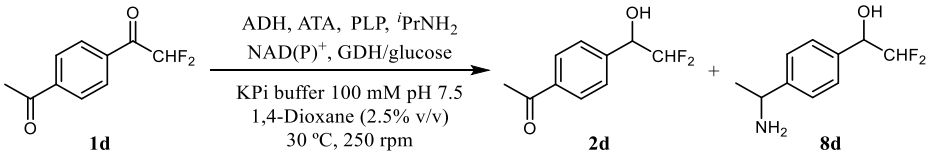
Entry	Substrate	ATA	*c* (%)[b]	*de* (%)[c]	*ee* (%)[c]	**8d**
1	(*R*)-**2d**	*ArS*TA	75	>99	>99	(*R*,*S*)
2		ATA-251	77	>99	>99	(*R*,*S*)
3		ATA-256	74	>99	>99	(*R*,*S*)
4		TA-P1-A06	24	>99	>99	(*R*,*S*)
5		ATA-024	50	>99	>99	(*R*,*R*)
6	(*S*)-**2d**	ATA-251	77	>99	>99	(*S*,*S*)
7		ATA-024	81	>99	>99	(*S*,*R*)

[a] Reaction conditions: **2d** (2.0 mg, 0.01 mmol), 1,4-dioxane (12.5 µL, 2.5% v/v), ATA (10.0 mg *ArS*TA or 2.0 mg Codexis ATA) and PLP (1 mM) in phosphate buffer (100 mM, pH 7.5, 500 µL total volume) containing isopropylamine (1.0 M) at 30 ºC and 250 rpm for 22 h in closed system.

[b] Percentage of products was measured by GC analysis of the reaction crude.

[c] Enantiomeric excess values were measured by HPLC after derivatization of the amino alcohol **8d** with Ac_2O and DMAP.

Table 5. Sequential and concurrent cascade approaches for the synthesis of amino alcohol **8d**.



Entry	Approach	ADH	ATA	**2d** (%)[a]	**8d** (%)[a]	*de* (%)[b]	*ee* (%)[b]
1	Sequential	*Ras*ADH	ATA-251	34	66 (*R*,*S*)	>99	>99
2		evo-1.1.200	ATA-251	13	87 (*S*,*S*)	>99	>99
3		*Ras*ADH	ATA-024	35	65 (*R*,*R*)	>99	>99
4		evo-1.1.200	ATA-024	11	89 (*S*,*R*)	>99	>99
5	Concurrent	*Ras*ADH	ATA-251	<1	>99 (*R*,*S*)	>99	>99
6		evo-1.1.200	ATA-251	<1	>99 (*S*,*S*)	>99	>99
7		*Ras*ADH	ATA-024	<1	>99 (*R*,*R*)	96[c]	>99
8		evo-1.1.200	ATA-024	<1	>99 (*S*,*R*)	96[d]	>99

[a] Percentage of products was measured by GC analysis of the reaction crude. The amino ketone **5d** was not found as reaction product in any case. Major amino alcohol **8d** stereoisomer appears in parentheses.

[b] Enantiomeric excess values were measured by HPLC after derivatization of the amino alcohol **8d** with Ac_2O and DMAP.

[c] 2% of (*R*,*S*)-**8d** diastereoisomer was found.

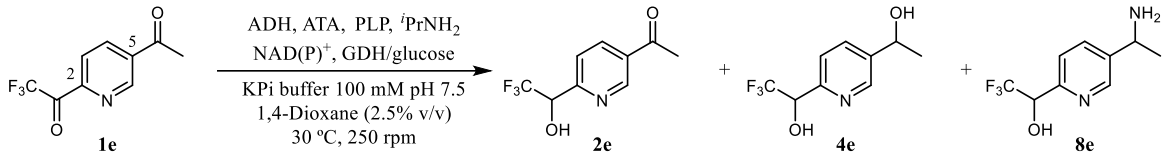
[d] 2% of (*R*,*R*)-**8d** diastereoisomer was found.

Broadening the substrate scope to pyridine derivatives

Finally, continuing our interest for the development of synthetic methods towards optically active (fluorinated) pyridine derivatives,[34] the preparation of diketones **1e** and **1f** was addressed (see Electronic Supporting Information). Next, ADHs (Tables S13 and S14) and ATAs (Tables S15 and S16) were screened to pick up the best enzyme candidates, searching the development of efficient multienzymatic reactions towards the formation of amino alcohols **8e** and **8f** (Tables 6 and 7).

Starting with the 1-(5-acetylpyridin-2-yl)-2,2,2-trifluoroethan-1-one (**1e**), a bioreduction screening study was performed employing 20 ADHs. Interestingly, 12 of these enzymes favoured the bioreduction of the trifluoroacetyl group, 3 of them mainly led to the monoreduction of the acetyl group, while complex mixtures were found for the other five cases including the appearance of the diol product **4e** (Table S13). On one hand, the (*R*)-selective *Ras*ADH and the (*S*)-selective KRED-P2-D11 and KRED-101 highly preferred the reduction of the trifluoroacetyl group (82-87%). On the other hand, the *Lb*ADH (88%) and the evo-1.1.200 (61%) favoured the chemoselective reduction of the methyl ketone. Regarding the biotransamination of **1e** (Table S15), both amino ketone enantiomers were exclusively isolated with high conversions, for instance the (*S*)-**5e** using overexpressed enzymes *Cv*TA (90%) or *ArS*TA (92%), and its antipode with the TA-P2-B01 (86%).

Table 6. Sequential and concurrent cascade approaches for the synthesis of amino alcohol **8e**.

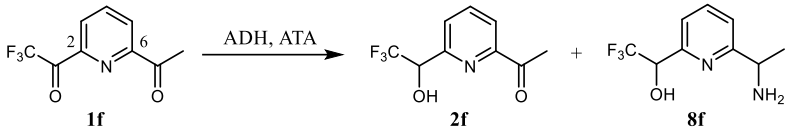


Entry	Approach	ADH	ATA	**2e** (%)[a]	**4e** (%)[a]	**8e** (%)[a]	*de* (%)[b]	*ee* (%)[b]
1	Sequential	*Ras*ADH	*ArS*TA	11	<1	89 (*R*,*S*)	98	>99
2		KRED-101	*ArS*TA	6	3	91 (*S*,*S*)	97	>99
3		*Ras*ADH	TA-P2-B01	25	<1	75 (*R*,*R*)	99	>99
4		KRED-101	TA-P2-B01	16	4	80 (*S*,*R*)	>99	>99
5	Concurrent	*Ras*ADH	*ArS*TA	20	1	79 (*R*,*S*)	98	>99
6		KRED-101	*ArS*TA	14	4	82 (*S*,*S*)	97	>99
7		*Ras*ADH	TA-P2-B01	14	<1	86 (*R*,*R*)	99	>99
8		KRED-101	TA-P2-B01	6	<1	94 (*S*,*R*)	>99	>99

[a] Percentage of products was measured by GC analysis of the reaction crudes. Major diastereoisomer in parentheses.

[b] Diastereomeric and enantiomeric excess values of the amino alcohol **8e** were measured by HPLC after acetylation with Ac_2O and DMAP.

Table 7. Sequential and concurrent cascade processes for the synthesis of amino alcohol **8f**.



Entry	1st step	2nd step	**2f** (%)[a]	**8f** (%)[a]	*de* (%)[b]	*ee* (%)[b]
1	KRED-P1-B02	ATA-251	1	99 (*S*,*S*)	99	>99
2	KRED-P1-B02	ATA-024	1	99 (*S*,*R*)	98	>99
3	ATA-251	KRED-P1-H08	<1	>99 (*R*,*S*)	50	>99
4	ATA-412	*Ras*ADH	<1	>99 (*R*,*R*)	82	>99
5	*Ras*ADH + *ArS*TA		4	96 (*RS*,*S*)	18[c]	>99/>99[d]
6	*Ras*ADH + ATA-024		3	97 (*RS*,*R*)	<1	92/>99[e]
7	KRED-P1-B02 + *ArS*TA		1	99 (*S*,*S*)	99	>99
8	KRED-P1-B02 + ATA-024		<1	>99 (*S*,*R*)	98	>99
8	KRED-P1-B05 + ATA-251		1	99 (*S*,*S*)	99	>99
9	KRED-P1-B05 + ATA-024		<1	>99 (*S*,*R*)	96	>99

[a] Percentage of products was measured by GC analysis of the reaction crude.

[b] Diastereomeric and enantiomeric excess values of amino ketone **8f** were measured by HPLC after derivatization with Ac_2O and DMAP. Major diastereoisomer appears in parentheses.

[c] The diastereosisomer (*S*,*S*)-**8f** was preferentially obtained instead of the (*R*,*S*)-**8f**.

[d] >99% *ee* of (*R*,*S*)-**8f**, >99% *ee* of (*S*,*S*)-**8f**.

[e] 92% *ee* of (*R*,*R*)-**8f**, >99% *ee* of (*S*,*R*)-**8f**.

Combining the best ADHs and ATAs, the bioreduction of **1e** was carried out with complementary *Ras*ADH and KRED-101 (Table S17), followed by the biotransamination of the resulting hydroxy ketone with the *ArS*TA and TA-P2-B01, obtaining the corresponding amino alcohol **8e** stereoisomers in good yields (73-87%) and excellent stereoselectivities (>96% *de* and >99% *ee*), observing in some cases the presence of the hydroxy ketone **2e** (0-14%) and the diol **4e** (4-6%). Ultimately, the best enzyme sets (*Ras*ADH/KRED-101 and *ArS*TA/TA-P2-B01) provided the corresponding amino alcohols **8e** through both sequential and concurrent approaches with excellent chemo- and stereoselectivity (80-94% yield, >96% *de* and >99% *ee*, Table 6).

Finally, when considering the pyridine derivative **1f** with both carbonyl groups at adjacent positions of the nitrogen atom, two important issues must be considered: (i) the transamination of **1f** towards **5f** should be more favourable as the resulting amine forms an intramolecular hydrogen bond with the pyridinic nitrogen, fact already observed in the biotransamination of 2-acetylpyridine derivatives;[34a] (ii) the bioreduction of the acetyl and trifluoracetyl groups are favoured as both the resulting hydroxy ketones can establish the intramolecular hydrogen bond with the nitrogen atom, which could negatively affect the discrimination between both carbonyl groups by the ADH.

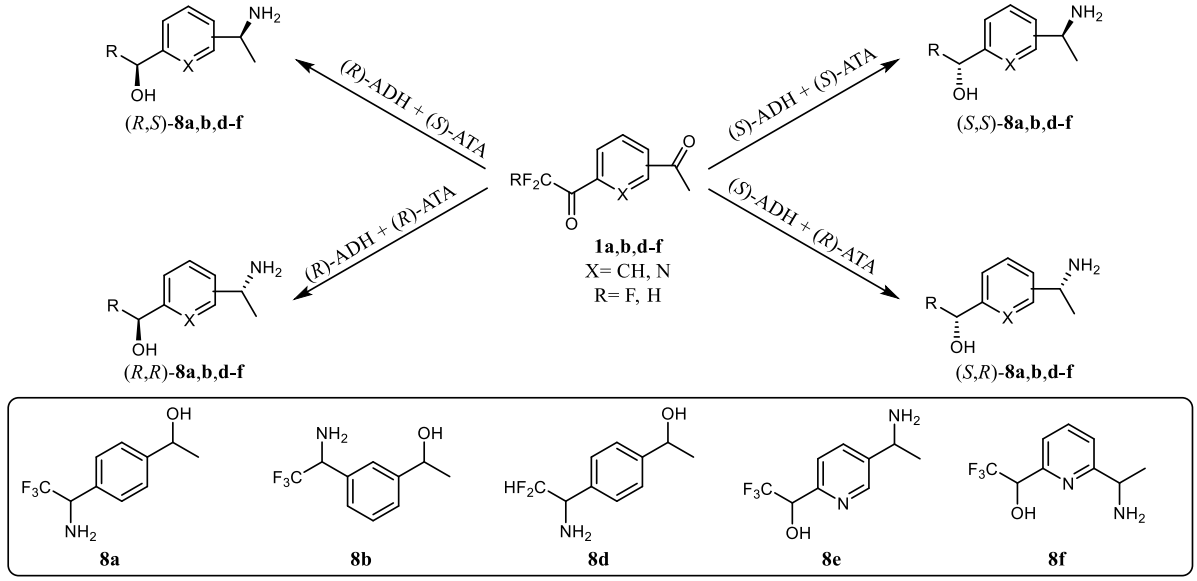
In this context, all the ADHs found to preferentially reduce the trifluoroacetyl group of **1f** displayed (*S*)-selectivity, even observing a change of enantiopreference with the *Ras*ADH, and identifying the KRED-P1-B02 and KRED-P1-B05 as suitable catalysts to obtain enantiopure (*S*)-**2f** (Table S14). Satisfyingly, all the tested ATAs showed excellent stereoselectivity and the preparation of both **5f** enantiomers was achieved with a quantitative conversion (Table S16).

Using the *ArS*TA and the ATA-303, the two **5f** enantiomers were individually obtained to study later the bioreduction of the trifluoroacetyl group in order to identify (*R*)-selective ADHs. As shown in Table S18, KRED-P1-H08 and *Ras*ADH allowed access to (*R*,*S*)-**8f** and (*R*,*R*)-**8f** from **5f** enantiomers, displaying both a good diastereoselectivity. Based on this results, both sequential and concurrent cascade transformation were developed (Table 7), performing the biotransamination as the first step of the sequential process for the synthesis of (*R*,*S*)- and (*R*,*R*)-**8f** diastereoisomers (entries 3-4), while (*S*,*S*)- and (*S*,*R*)-**8f** were obtained following the conventional sequence (entries 1-2) or the concurrent cascade approach (entries 7-10) with excellent selectivities.

Conclusion

Overall, it can be concluded that after synthesizing a series of (het)aryl unsymmetrical diketones their bioreduction and biotransamination reactions has been extensively investigated, finding adequate procedures for the synthesis of the corresponding amino alcohol stereoisomers with high chemo- and stereocontrol. On one hand, di- and trifluoroacetyl groups were selectively reduced by using a variety of ADHs with (*S*)- and (*R*)-selectivity, requiring the use of only one equivalent of glucose to avoid the formation of the corresponding diol. On the other hand, the biotransamination of the acetyl groups was significantly preferred over the modification of trifluoroacetophenones. This opposite selectivity displayed by ADHs and ATAs was exploited in order to synthesise a panel of amino alcohols from the corresponding diketones developing a chemoenzymatic cascade process. In the Table 8 a summary of the best results obtained is depicted, where the four possible stereoisomers of **8a,b,d-f** were obtained with excellent diastereo- and enantioselectivities by the combination of the proper biocatalysts in both sequential or concurrent approaches.

Table 8. Summary of the results obtained for the sequential and concurrent cascade synthesis of **8a**,**b**,**d-f** stereoisomers.



Substrate	ADH/ATA	**8a,b,d-f**	Sequential			Concurrent		
			c (%)[a]	*de* (%)[b]	*ee* (%)[b]	*c* (%)[a]	*de* (%)[b]	*ee* (%)[b]
1a (R= CF_3) (X= CH)	*Ras*ADH/*Ar*STA	(*R*,*S*)	93	98	>99	95	98	>99
	*Ras*ADH/ATA-024	(*R*,*R*)	96	98	>99	98	98	>99
	evo-1.1.200/*Ar*STA	(*S*,*S*)	90	97	>99	97	97	>99
	evo-1.1.200/ATA-024	(*S*,*R*)	96	>99	>99	>99	>99	>99
1b (R= CF_3) (X= CH)	KRED-NADH-101/*Ar*STA	(*R*,*S*)	92	>99	>99	80	>99	>99
	KRED-NADH-101/ATA-303	(*R*,*R*)	78	>99	>99	90	>99	>99
	evo-1.1.200/*Ar*STA	(*S*,*S*)	95	>99	>99	62	>99	>99
	evo-1.1.200/ATA-303	(*S*,*R*)	92	>99	>99	96	>99	>99
1d (R= CHF_2) (X= CH)	*Ras*ADH/ATA-251	(*R*,*S*)	66	>99	>99	>99	>99	>99
	*Ras*ADH/ATA-024	(*R*,*R*)	65	>99	>99	>99	>99	>99
	evo-1.1.200/ATA-251	(*S*,*S*)	87	>99	>99	>99	96	>99
	evo-1.1.200/ATA-024	(*S*,*R*)	89	>99	>99	>99	96	>99
1e (R= CF_3) (X= N)	*Ras*ADH/*Ar*STA	(*R*,*S*)	89	98	>99	79	98	>99
	*Ras*ADH/TA-P2-B01	(*R*,*R*)	75	97	>99	86	97	>99
	KRED-101/*Ar*STA	(*S*,*S*)	91	99	>99	82	99	>99
	KRED-101/TA-P2-B01	(*S*,*R*)	80	>99	>99	94	>99	>99
1f (R= CF_3) (X= N)	*Ras*ADH/*Ar*STA	(*R*,*S*)	>99	34	>99	96	18[c]	>99
	*Ras*ADH/ATA-024	(*R*,*R*)	>99	82	>99	97	<1	92
	KRED-P1-B02/*Ar*STA	(*S*,*S*)	99	99	>99	99	99	>99
	KRED-P1-B02/ATA-024	(*S*,*R*)	99	98	>99	>99	98	>99

[a] Conversion values measured by GC analysis of the reaction crude.

[b] Diastereomeric and enantiomeric excess values calculated by HPLC or GC after derivatization of the corresponding amino alcohol **8a**,**b,d-f** with Ac_2O and DMAP.

[c] The diastereosisomer (*S*,*S*)-**8f** was preferentially obtained instead of the (*R*,*S*)-**8f**.

Experimental Section

Made in house ADHs overexpressed in *E. coli* and later lyophilized (*Ras*ADH, *Sy*ADH, ADH-T, *Lb*ADH, *Te*SADH and ADH-A) were obtained as previously reported in the literature,[21-26] while evo-1.1.200 ADH was acquired from Evoxx technologies GmbH. Glucose dehydrogenase (GDH-105) was obtained from Codexis Inc. Lyophilized made in house ATAs overexpressed in *E. coli* *Cv*TA, *Ar*STA, *Ar*RTA and its mutant *ArRmut11* were obtained as already described,[28-31] while commercial ATAs were acquired from Codexis Inc. D-Glucose, NADPH, NADH and all other reagents for chemical transformations and product isolation/purifications were obtained from Sigma-Aldrich and used as received.

Thin layer chromatographies (TLCs) were conducted with silica gel precoated plates and visualized with UV and potassium permanganate stain. Column chromatographies were performed using silica gel (230-400 mesh). NMR spectra were recorded on a 300 MHz spectrometer. All chemical shifts (δ) are given in parts per million (ppm) and referenced to the residual solvent signal as internal standard. IR spectra were recorded on a spectrophotometer on NaCl pellets.

Gas chromatography (GC) analyses were performed on Hewlett-Packard 6860 chromatograph equipped with a FID detector using the columns HP-1 and CP-Chirasil-Dex CB. High performance liquid chromatography (HPLC) analyses were carried out in an Agilent 1260 Infinity chromatograph with UV detector at a 210 nm wavelength using the chiral

columns Chiralcel OJ-H, Chiralpak AD-H, Chiralpak IA, Chiralpak IC and Chiralpak ID (see section VII of the SI for details). Non-chiral GC analyses served to calculate conversion and chiral GC and HPLC analyses were used to measure the enantiomeric excess (*ee*) and diastereomeric excess (*de*) values. For this purpose, *in situ* derivatizations were carried out by addition of 10 µL of acetic anhydride and a spoon of DMAP (for alcohols and amino alcohols) or K_2CO_3 (for amines) to 500 µL of the corresponding EtOAc-extracted reaction and were stirred at room temperature for 1 h at 720 rpm. The acetylated samples were washed with water (500 µL), dried over Na_2SO_4 and analyzed by GC or HPLC.

Measurement of the optical rotation was carried out at 590 nm in a standard polarimeter.

Absolute configurations of hydroxy ketones **2a-f**, amino ketones **5a-f** and amino alcohols **8a-f** were assigned based on the known selectivity displayed by the enzymes. Additionally, the retention order of the enantiomers of compound **2b** in the CP-Chirasil-Dex CB column is in agreement with the reported by Grau and coworkers.[19]

Typical procedure for the bioreduction of diketones 1a-f in analytical scale. Diketone **1a-f** (0.01 mmol, 20 mM) was dissolved in 1,4-dioxane (12.5 µL, 2.5% v/v) inside an Eppendorf tube. Then, a phosphate buffer (100 mM, pH 7.5, 500 µL total volume), glucose (20 mM), GDH (0.05 mg), $NAD(P)^+$ (1 mM) and the corresponding ADH (6.0 mg *E. coli* overexpressed ADH, 0.06 mg evo-1.1.200 or 2.0 mg Codexis KRED) were added. The mixture was shaken at 250 rpm and 30 ºC, and after the required time, the reaction was extracted with EtOAc (3 x 500 µL). The organic phases were combined and washed with water (500 µL), dried over Na_2SO_4 and analyzed by GC and/or HPLC.

Typical procedure for the semi-preparative bioreduction of diketone 1a towards the synthesis of 2a enantiomers. The *para*-(trifluoroacetyl)acetophenone (**1a**, 21.6 mg, 0.1 mmol) was dissolved in 1,4-dioxane (125 µL, 2.5% v/v) inside a Falcon tube. Then, a phosphate buffer (100 mM, pH 7.5, 4.9 mL), glucose (18.0 mg, 0.1 mmol), GDH (0.1 mg) and the corresponding enzyme-cofactor [RasADH (5.0 mg) and $NADP^+$ (1 mM); or evo-1.1.200 (1.0 mg) and NAD^+ (1 mM)] for accessing to the (*R*) and (*S*)-**2a**, respectively. The mixture was shaken at 250 rpm and 30 ºC during 2 h, and after this time the reaction was extracted with EtOAc (3 x 5 mL). The organic phases were combined and washed with water (5 mL), dried over Na_2SO_4, and filtered. The solvent was distilled under reduced pressure yielding the hydroxy ketones (*R*) or (*S*)-**2a** as yellow solids (99% isolated yield). Similarly, hydroxy ketones **2b**,**d-f** could be obtained following an identical procedure.

1-[4-(2,2,2-Trifluoro-1-hydroxyethyl)phenyl]ethan-1-one (2a). Colorless oil (22 mg, 99%). ^{1}H-NMR (THF-d_8, 300.13 MHz): δ 2.56 (s, 3H), 3,60 (brs, 1H), 5.09-5.22 (m, 1H), 6.11 (d, J_{HH} = 5.1 Hz, 1H), 7.63 (d, J_{HH} = 8.3 Hz, 2H), 7.75-7.80 (dt, J_{HH} = 8.4, 1.7 Hz, 2H) ppm. ^{13}C-NMR (THF-d_8, 75.5 MHz): δ 26.5 (CH_3), 72.3 (q, $^2J_{CF}$ = 31.1 Hz, CH), 125.9 (q, $^1J_{CF}$ = 282.6 Hz, C), 128.7 (2CH), 129.0 (2CH), 138.9 (C), 141.7 (C), 196.9 (C) ppm. ^{19}F-NMR (THF-d_8, 282.35 MHz): δ –78.94 (d, $^3J_{HF}$ = 7.0 Hz) ppm.

1-[4-(2,2-Difluoro-1-hydroxyethyl)phenyl]ethan-1-one (2d). Colorless oil (19 mg, 95%). ^{1}H-NMR ($CDCl_3$, 300.13 MHz): δ 2.59 (s, 3H), 3.50 (brs, 1H), 4.90 (td, J_{HF} = 10.3 Hz, J_{HH} = 4.5 Hz, 1H), 5.77 (td, J_{HF} = 55.8 Hz, J_{HH} = 4.5 Hz, 1H), 7.52 (d, J_{HH} = 8.2 Hz, 2H), 7.94 (d, J_{HH} = 8.4 Hz, 2H) ppm. ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ 26.8 (CH_3), 73.2 (t, $^2J_{CF}$ = 24.6 Hz, CH), 115.6 (t, $^1J_{CF}$ = 246.0 Hz, CH), 127.5 (2CH), 128.7 (2CH), 137.4 (C), 141.2 (C), 198.4 (C) ppm. ^{19}F-NMR ($CDCl_3$, 282.35 MHz): δ –128.01 (ddd, $^2J_{FF}$ = 285.2 Hz, $^2J_{HF}$ = 55.6 Hz, $^3J_{HF}$ = 9.7 Hz), –126.79 (ddd, $^2J_{FF}$ = 285.1 Hz, $^2J_{HF}$ = 56.1 Hz, $^3J_{HF}$ = 10.8 Hz) ppm.

Typical procedure for the biotransamination of diketones 1a-f or hydroxyketones 2a-f in analytical scale. Diketone **1a-f** (0.01 mmol, 20 mM) or hydroxy ketone **2a-f** (0.01 mmol, 20 mM) was dissolved in 1,4-dioxane (12.5 µL, 2.5% v/v) inside an Eppendorf tube. Then, a phosphate buffer (100 mM, pH 7.5, total volume 500 µL) containing ^{i}PrNH$_2$ (1.0 M) and PLP (1 mM) were added, following by the addition of the corresponding ATA (10.0 mg *E. coli* overexpressed ATA or 2.0 mg Codexis ATA). The mixture was shaken at 250 rpm and 30 ºC and, after the required time, the reaction was stopped by addition of an aqueous NaOH 4 M solution (200 µL) and extracted with EtOAc (3 x 500 µL). The organic phases were combined and washed with water (500 µL), dried over Na_2SO_4, and analyzed by GC and/or HPLC.

Typical procedure for the semi-preparative biotransamination of diketone 1a towards the synthesis of 5a enantiomers. Diketone **1a-f** (30.2 mg, 0.14 mmol) was dissolved in 1,4-dioxane (175 µL, 2.5% v/v) inside an Eppendorf tube. Then, a phosphate buffer (100 mM, pH 7.5, total volume 7.0 mL) containing ^{i}PrNH$_2$ (1.0 M) and PLP (1 mM) were added, following by the addition of the corresponding ATA [50.0 mg *Ar*STA or 20 mg ATA-024 for the preparation of (*S*) and (*R*)-**5a**, respectively]. The mixture was shaken at 250 rpm and 30 ºC for 48 h without closing the tube's lid, just some cotton was put in the upper part. The reaction was stopped by addition of an aqueous NaOH 10 M solution (0.5 mL) and extracted with EtOAc (3 x 5 mL). The organic phases were combined and washed with water (5 mL), dried over Na_2SO_4, and filtered. After solvent distillation under reduced pressure, amino ketones (*S*)-**5a** (28 mg, 93% isolated yield) or (*R*)-**5a** (26 mg, 85% isolated yield) were recovered.

1-[4-(1-Amino-2,2,2-trifluoroethyl)phenyl]ethan-1-one (5a). Yellowish solid (26-28 mg, 85-93%). ^{1}H-NMR (THF-d_8, 300.13 MHz): δ 1.41 (d, q, J_{HH} = 6.7 Hz, 3H), 3.18 (brs, 2H), 4.23 (q, J_{HH} = 6.6 Hz, 1H), 7.54 (d, J_{HH} = 8.0 Hz, 2H), 8.03 (d, J_{HH} = 8.0 Hz, 2H) ppm. ^{13}C-NMR (THF-d_8, 75.5 MHz): δ 25.3 (CH_3), 51.3 (CH), 116.8 (q, $^1J_{CF}$ = 291.5 Hz, C), 126.7 (2CH), 128.8 (C), 130.7 (2CH), 155.3 (C), 180.2 (q, $^2J_{CF}$ = 35.3 Hz, C) ppm. ^{19}F-NMR (THF-d_8, 282.35 MHz): δ –71.40 ppm.

Typical procedure for the synthesis of amino alcohols 8a,b,d-f through a sequential cascade in analytical scale. Diketone **1a,b,d-f** (0.02 mmol, 20 mM) was dissolved in 1,4-dioxane (25 µL, 2.5% v/v) followed by the addition of a phosphate buffer (100 mM, pH 7.5, 1.0 mL) containing ^{i}PrNH$_2$ (1.0 M), the corresponding ADH (6.0 mg *Ras*ADH, 0.06 mg evo-1.1.200 or 2.0 mg Codexis KRED), $MgSO_4$ (2 mM only for the commercial ADHs), $NAD(P)^+$ (0.5 mM), GDH (0.1 mg) and glucose (20 mM). The mixture was shaken at 30 ºC and 250 rpm for 24 h. After this time, PLP (1 mM) and the corresponding ATA (20 mg *Ar*STA or 10.0 mg Codexis ATA) was added and shaken for additional 24 h in the same reaction conditions (30 ºC and 250 rpm). The system was open to air for substrates **1a**,**b**,**d**, and the reaction stopped by addition of an aqueous NaOH solution (4 M, 200 µL for **1a**,**b**,**d**; 1 M, 100 µL for **1e**,**f**). The mixture was extracted with EtOAc (3 x 500 µL), and then combined organic phases washed with water (500 µL), dried over Na_2SO_4 and analyzed by GC and/or HPLC.

Typical procedure for the synthesis of amino alcohols 8a,b,d-f through a concurrent cascade approach in analytical scale. A solution of the corresponding diketone **1a,b,d-f** (0.01 mmol, final concentration of 10 mM) in 1,4-dioxane (25 µL, 2.5% v/v) was added over a suspension of the corresponding ADH (10.0 mg *Ras*ADH or 1.0 mg evo-1.1.200) in a phosphate buffer (100 mM, pH 7.5, total volume 0.5 mL) containing glucose (20 mM), GDH (0.1 mg), $MgSO_4$ (2 mM only for the commercial ADHs) and $NAD(P)^+$ (0.5 mM). Finally, the corresponding ATA (10.0 mg *Ar*STA or 5.0 mg Codexis ATA) and a phosphate buffer (100 mM, pH 7.5, 0.5 mL) containing ^{i}PrNH$_2$ (1.0 M) and PLP (1 mM) were added. The mixture was shaken at 30 ºC and 250 rpm for 48 h, maintaining the system

open to air for substrates **1a**,**b**,**d**, and the reaction stopped by addition of an aqueous NaOH solution (4 M, 200 µL for **1a**,**b**,**d**; 1 M, 100 µL for **1e**,**f**). The mixture was extracted with EtOAc (3 x 500 µL), and then combined organic phases washed with water (500 µL), dried over Na_2SO_4 analyzed by GC and/or HPLC.

Typical procedure for the synthesis of amino alcohols 8a,b,d-f through a concurrent cascade approach in semi-preparative scale. As a representative example, for the synthesis of (*R*,*R*)-**8a** a solution of the diketone **1a** (30 mg, 0.14 mmol) in 1,4-dioxane (175 µL, 2.5% v/v) was added over a suspension of *Ras*ADH (15 mg) in a phosphate buffer (100 mM, pH 7.5, total volume 3.0 mL) containing glucose (25 mg, 0.14 mmol), GDH (0.1 mg) and $NADP^+$ (2.5 mM). After incubation of the mixture at room temperature for 15 minutes, the ATA-024 (30 mg) and a phosphate buffer (100 mM, pH 7.5, 7.0 mL) containing $^iPrNH_2$ (1.0 M) and PLP (1 mM) were added. The mixture was shaken at 30 ºC and 250 rpm for 48 h, maintaining the system open to air. The reaction was stopped by addition of an aqueous NaOH 4 M solution (1 mL) and extracted with EtOAc (3 x 5 mL). Then, the organic phases were combined and an aqueous HCl 1 M solution (10 mL) was added. After two extractions with EtOAc (2 x 10 mL), the aqueous phase was basified by addition of an aqueous NaOH 10 M solution (2 mL), which was extracted with EtOAc (3 x 10 mL), dried over Na_2SO_4, and filtered. After distillation of the solvent under reduced pressure, the amino alcohol (*R*,*R*)-**8a** was isolated as a white solid (30 mg, 99% isolated yield).

1-[4-(1-Aminoethyl)phenyl]-2,2,2-trifluoroethan-1-ol (8a). White solid (30 mg, 99%). Mp: 149-150 ºC (*R*,*R*); 152-154 ºC (*R*,*S*). ^{1}H-NMR (THF-d_8, 300.13 MHz)**:** δ 1.36 (d, J_{HH} = 6.6 Hz, 3H), 4.05 (brs, 3H), 4.12 (q, J_{HH} = 6.6 Hz, 1H), 4.98 (q, J_{HF} = 7.2 Hz, 1H), 7.42 (s, 4H) ppm. ^{13}C-NMR (THF-d_8, 75.5 MHz): δ 25.5 (CH_3), 52.2 (CH), 72.6 (q, $^2J_{CF}$ = 30.9 Hz, CH), 126.3 (q, $^1J_{CF}$ = 282.4 Hz, C), 126.8 (2CH), 128.6 (2CH), 135.8 (C), 148.7 (C) ppm. ^{19}F-NMR (THF-d_8, 282.35 MHz)**:** δ –79.02 (d, $^3J_{HF}$ = 7.2 Hz) ppm. $[\alpha]_D^{20}$= –14.4 [*c* 1, MeOH, >99% *ee* (*R*,*R*)-**8a**); –38.9 (*c* 0.4, MeOH, >99% *ee* (*R*,*S*)-**8a**].

1-[3-(1-Aminoethyl)phenyl]-2,2,2-trifluoroethan-1-ol (8b). Yellowish solid (29 mg, 95%). Mp: 86-98 ºC. ^{1}H-NMR (THF-d_8, 300.13 MHz): δ 1.28 (d, J_{HH} = 6.7 Hz, 3H), 1.29 (brs, 2H), 3.10 (brs, 1H), 4.06 (q, J_{HH} = 6.6 Hz, 1H), 4.97 (q, J_{HF} = 7.2 Hz, 1H), 7.22-7.41 (m, 3H), 7.50 (s, 1H) ppm. ^{13}C-NMR (THF-d_8, 75.5 MHz): δ 26.5 (CH_3), 52.3 (CH), 72.9 (q, $^2J_{CF}$ = 30.9 Hz, CH), 126.2 (q, $^1J_{CF}$ = 283.1 Hz, C), 126.2 (CH), 126.6 (CH), 127.1 (CH), 128.8 (CH), 137.0 (C), 149.6 (C) ppm. ^{19}F-NMR (THF-d_8, 282.35 MHz): δ –78.99 (d, $^3J_{HF}$ = 7.3 Hz) ppm. $[\alpha]_D^{20}$= +23.3 [*c* 0.4, MeOH, >99% *ee* (*S*,*R*)-**8b**].

1-[4-(1-Aminoethyl)phenyl]-2,2-difluoroethan-1-ol (8d). White solid (21 mg, 75%). Mp: 116-117 ºC. ^{1}H-NMR (THF-d_8, 300.13 MHz): δ 1.30 (d, J_{HH} = 6.5 Hz, 3H), 3.36 (brs, 3H), 4.06 (q, J_{HH} = 6.6 Hz, 1H), 4.66 (ddd, J_{HF} = 11.4, 9.9 Hz, J_{HH} = 4.7 Hz, 1H), 5.71 (td, J_{HF} = 56.4 Hz, J_{HH} = 4.7 Hz, 1H), 7.28-7.43 (m, 4H) ppm. ^{13}C-NMR (THF-d_8, 75.5 MHz): δ 26.3 (CH_3), 52.2 (CH), 73.8 (t, $^2J_{CF}$ = 24.3 Hz, CH), 117.9 (t, $^1J_{CF}$ = 245.0 Hz, CH), 126.6 (2CH), 128.2 (2CH), 137.5 (C), 149.2 (C) ppm. ^{19}F-NMR (THF-d_8, 282.35 MHz): δ –128.42 (ddd, $^2J_{FF}$ = 280.6 Hz, $^2J_{HF}$ = 56.0 Hz, $^3J_{HF}$ = 9.9 Hz), –126.91 (ddd, $^2J_{FF}$ = 280.6 Hz, $^2J_{HF}$ = 56.9 Hz, $^3J_{HF}$ = 11.6 Hz) ppm. $[\alpha]_D^{20}$= +4.0 [*c* 0.4, $CHCl_3$, >99% *ee* (*R*,*R*)-**8d**].

1-[5-(1-Aminoethyl)pyridin-2-yl]-2,2,2-trifluoroethan-1-ol (8e). Viscous white-yellow solid (26 mg, 88%). ^{1}H-NMR (THF-d_8, 300.13 MHz): δ 1.33 (d, J_{HH} = 6.6 Hz, 3H), 3.40 (brs, 3H), 4.15 (q, J_{HH} = 6.6 Hz, 1H), 5.05 (q, J_{HF} = 7.1 Hz, 1H), 7.51 (d, J_{HH} = 8.1 Hz, 1H), 7.85 (dd, J_{HH} = 8.2, 2.3 Hz, 1H), 8.55 (d, J_{HH} = 2.3 Hz, 1H) ppm. ^{13}C-NMR (THF-d_8, 75.5 MHz)**:** δ 26.1 (CH_3), 49.8 (CH), 73.2 (q, $^2J_{CF}$ = 30.7 Hz, CH), 122.7 (CH), 125.9 (q, $^1J_{CF}$ = 283.0 Hz, C), 135.1 (CH), 144.9 (C), 147.9 (CH), 153.7 (C) ppm. ^{19}F-NMR (THF-d_8, 282.35 MHz): δ –78.53 (d, $^3J_{HF}$ = 7.1 Hz) ppm. $[\alpha]_D^{20}$= +21.8. [*c* 0.4, MeOH, >99% *ee* (*S*,*R*)-**8e**].

1-[6-(1-Aminoethyl)pyridin-2-yl]-2,2,2-trifluoroethan-1-ol (8f). White solid (27 mg, 90%). Mp: 92-94 ºC. ^{1}H-NMR ($CDCl_3$, 300.13 MHz): δ 1.46 (d, J_{HH} = 6.6 Hz, 3H), 3.92 (brs, 3H), 4.24 (q, J_{HH} = 6.6 Hz, 1H), 5.02 (q, J_{HF} = 6.8 Hz, 1H), 7.29 (d, J_{HH} = 7.7 Hz, 1H), 7.33 (dd, J_{HH} = 7.8 Hz, 1H), 7.72 (t, J_{HH} = 7.7 Hz, 1H) ppm. ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ 24.0 (CH_3), 52.0 (CH), 71.1 (q, $^2J_{CF}$ = 31.2 Hz, CH), 121.0 (CH), 121.1 (CH), 124.3 (q, $^1J_{CF}$ = 283.2 Hz, C), 138.1 (CH), 151.3 (CH), 163.4 (C) ppm. ^{19}F-NMR ($CDCl_3$, 282.35 MHz): δ –77.82 (d, $^3J_{HF}$ = 6.7 Hz) ppm. $[\alpha]_D^{20}$= +14.4 [*c* 0.5, MeOH, >99% *ee* (*S*,*R*)-**8f**].

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