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Enzymatic Reduction: a Useful Tool to Access Enantioenriched Fluorinated Molecules under Sustainable Conditions

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Abstract. We report here a mild and efficient process to access diversely functionalized enantioenriched β -*gem*-difluorinated alcohols by using biocatalytic reduction in yields up to 99% and enantiomeric excesses up to >99%. This novel approach involves enzymatic reduction of α -*gem*-difluorinated heterocyclic and carbocyclic ketone derivatives as well as an acyclic *gem*-difluorinated β -ketosulfone and proceeds under mild conditions at room temperature under aqueous conditions to produce a range of high added value enantioenriched difluorinated alcohols including 3,3-difluorochromanol, 3,3-difluorothiochromanol, 3,3-difluoro-dihydroquinolinol, and α -difluorinated β -hydroxysulfone derivatives. A comparative study between enzymatic reduction and metal-catalyzed asymmetric transfer hydrogenation was carried out as well.

Keywords: Fluorine; Biocatalysis; Enzymatic reduction; Alcohols; Heterocycles; Carbocycles

Because fluorination of organic compounds often profoundly affects their physical and chemical properties, particularly their metabolic stability and lipophilicity, the access to fluorine-containing molecules, especially chiral fluorinated products, constitutes an active research area in the field of agrochemicals or pharmaceutical industry.^[1-7] Many examples of drug candidates involve the replacement of a hydrogen atom by a single fluorine atom or a CF₃ group, but the introduction of a geminal difluoromethyl group has also been successfully exploited to improve the performances of the corresponding active compounds.^[8-13] Consequently, a variety of geminal difluoromethylated bioactive molecules or drugs have been developed in the past decades (Figure 1).^[14-18] In that context, we have

previously reported an access to valuable enantioenriched β -hydroxy *gem*-difluorinated heterocyclic and acyclic derivatives through Rh(III)-catalyzed asymmetric transfer hydrogenation of the corresponding ketones.^[19] In line with our ongoing efforts directed towards the synthesis of enantioenriched fluorinated compounds^[20] and aiming at developing an even greener procedure for the preparation of these high added value building blocks, we now investigated a biocatalytic approach by performing an enzymatic ketone reduction,^[21] thus reducing the environmental footprint by avoiding the use of metal-based catalysts as well as organic solvents. Another main advantage of enzymes resides in their high specific recognition for the substrates allowing for not only high control of the stereochemistry but also minimal side reactions and by-products, making the purification process easier.^[22-28]

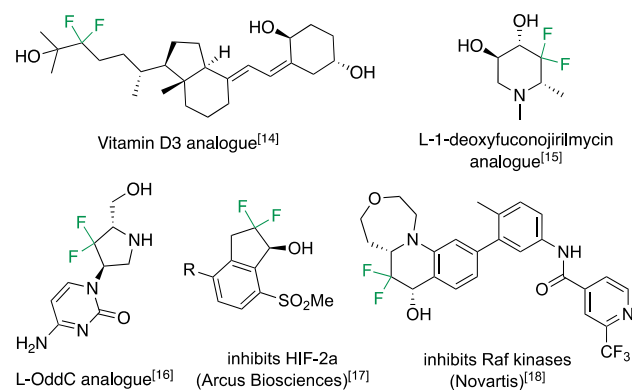
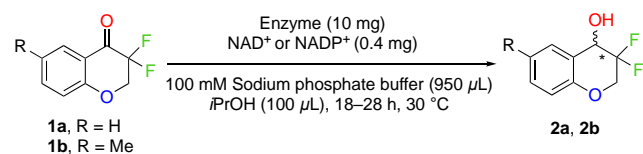


Figure 1. Examples of chiral bioactive *gem*-difluorinated compounds

We report herein an efficient enzymatic reduction of α -gem-difluorinated heterocyclic and carbocyclic ketone derivatives as well as an acyclic gem-difluorinated β -ketosulfone, that has never been reported to date, by using a selection of alcohol dehydrogenases provided by Protéus by SEQENS (Seqenzym[®] kits).^[29]

We first examined the enzymatic reduction of 3,3-difluorochroman-4-one **1a** and 3,3-difluoro-6-methylchroman-4-one **1b** with the Seqenzym[®] kit provided by Protéus by SEQENS and relying on a library of seven enzymes E4337, E4336, E4391, E3373, E3653, E4410 and E4408 (Table 1). In order to select the best enzyme candidate, the enzyme screening approach was combined with appropriate reaction conditions. The biocatalysis was carried out using 5 mg of the ketone substrate (0.027 M) with 5 mg of enzyme (cell-free extracts) as a catalyst, in the presence of NAD⁺ or NADP⁺ depending on the enzyme and 100 μ L of *i*PrOH as the cosubstrate for cofactor regeneration in 950 μ L of a 100 mM sodium phosphate buffer (pH 7) weekly prepared. With the enzyme E4337, a conversion of only 49% was observed for the reduction of **1a** that delivered the corresponding alcohol **2a** as the (*R*) enantiomer with an enantiomeric excess of 80.7% (Table 1, entry 1).

Table 1.



Entry	Ketone	Enzyme	Conversion (%) ^[a]	ee (%) ^[b]	(<i>R</i>)/(<i>S</i>)
1	1a	E4337	49	80.7	(<i>R</i>)
2	1b		0	–	–
3	1a	E4336	100	0.8	(<i>S</i>)
4	1b		100	14.9	(<i>S</i>)
5	1a	E4391	100	3.8	(<i>S</i>)
6	1b		100	98.4	(<i>S</i>)
7	1a	E3373	100	33	(<i>S</i>)
8	1b		100	99.2	(<i>S</i>)
9	1a	E3653	100	99.8	(<i>R</i>)
10	1b		100	35.8	(<i>R</i>)
11	1a	E4410	100	97.7	(<i>S</i>)
12	1b		100	99.9	(<i>S</i>)
13	1a	E4408	100	99.5	(<i>S</i>)
14	1b		100	99.9	(<i>S</i>)

^[a] The reaction was carried out on 5 mg of ketone. The conversion was determined by ¹H NMR of the crude after extraction of the reaction mixture with AcOEt.

^[b] Enantiomeric excess was determined by SFC analysis with a chiral stationary phase.

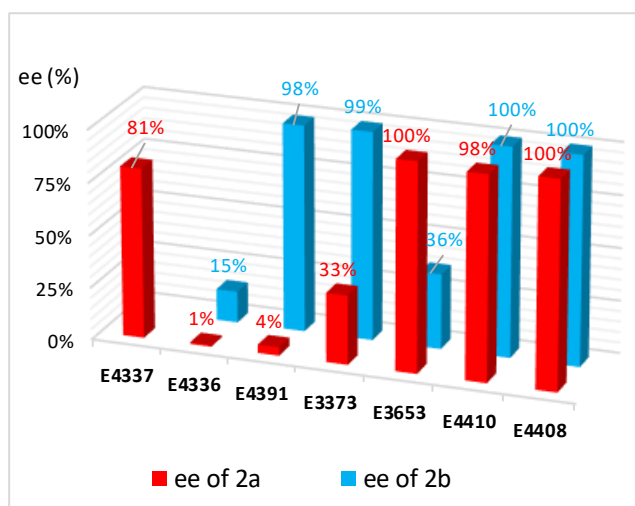


Figure 2.

The same conditions applied to ketone **1b** however failed to give any conversion (Table 1, entry 2). A full conversion was reached with E4336 for both **1a** and **1b** but the reaction provided low enantioselectivities of 0.8% and 14.9% respectively, in favor of the (*S*) enantiomer (Table 1, entries 3–4). The use of E4391 allowed access to the corresponding alcohol (*S*)-**2a** in 3.8% ee and (*S*)-**2b** in 98.4% ee (Table 1, entries 5–6). Comparable results were observed with E3373 that delivered enantioselectivities of 33% and 99.2% ee, respectively for (*S*)-**2a** and (*S*)-**2b** (Table 1, entries 7–8). A higher ee of 99.8% was observed for the alcohol (*R*)-**2a** with E3653 that provided (*R*)-**2b** in only 35.8% ee (Table 1, entries 9–10). Finally, enzymes E4410 and E4408 allowed a high enantioinduction with ee values ranging from 97.7% to 99.9% for both substrates **1a** and **1b**. Indeed, enantioselectivities of 99.5% and 99.9% ee were obtained with E4410 for (*S*)-**2a** and (*S*)-**2b** (Table 1, entries 11–12) whereas E4408 furnished these alcohols in 99.5% and 99.9% ee (Table 1, entries 13–14). A comparison of the catalytic performances of the seven enzymes examined for the reduction of substrates **1a** and **1b** is shown in Figure 2.

Considering the high performances observed with the enzyme E4408 for the reduction of both **1a** and **1b**, and encouraged by these initial results, we next investigated the scope of the reaction by using this enzyme and by increasing the substrate loading from 5 mg to 20 mg while decreasing the enzyme loading from 10 mg to 5 mg. The quantity of *i*PrOH was also reduced and adjusted to still remain in large excess to shift the equilibrium towards the formation of the desired alcohol (Table 2). With these new set of conditions in hands, a series of variously substituted 3,3-difluorochroman-4-ones **1a–1h** having on the benzene ring electron-donating methyl or methoxy groups, and electron-withdrawing substituents such as nitro, bromine, chlorine, or fluorine substituents, were tested for this reaction.

Table 2. Scope of the enzymatic reduction.

R - **1a–1q** or **1r**

Enzyme E4408 (5 mg)
 NAD⁺ (0.4 mg)
 100 mM Sodium phosphate buffer
 iPrOH (200 μL)
 19–26 h, 30 °C

R - **2a–2q** or **2r**

X = O, S, NBoc, CH₂
 n = 0, 1

Entry	Product	Yield (%) ^[a]	ee (%) ^[b]	Entry	Product	Yield (%) ^[a]	ee (%) ^[b]
1	2a	quant.	>99	11	2k	quant.	>99
2	2b	quant.	99	12	2l	quant.	>99
3	2c	quant.	>99	13	2m	quant.	>99
4	2d	quant.	>99	14	2n	quant.	>99
5	2e	quant.	>99	15	2o	quant.	>99
6	2f	quant.	>99	16	2p	quant.	>99
7	2g	quant.	>99	17	2q	quant.	>99
8	2h	quant.	>99	18	2r	quant.	98
9	2i	quant.	>99		2i (CCDC 2342545)		
10 ^[c]	2j	quant.	96		2q (CCDC 2342547)		

^[a] The reaction was conducted on 20 mg of the ketone **1**. Isolated yields. ^[b] Enantiomeric excess was determined by SFC analysis with a chiral stationary phase. ^[c] The reduction was conducted with enzyme E3653 on 10 mg of the ketone.

All substrates afforded a complete conversion with quantitative yields and ee values of 99% or >99% for alcohols **2a–2h** attesting to the effectiveness of the process regardless of the nature of the substituent on

the aromatic ring (Table 2, entries 1–8). A difluorothiochromanone was also examined and 3,3-difluorothiochroman-4-one gave comparable results with quantitative yield and >99% ee (Table 2, entry 9).

However, the same conditions applied to the 3,3-difluoro-dihydroquinoline derivative **1j** failed to yield a full conversion. Nevertheless, a quantitative yield of **2j** was obtained by using the enzyme E3653 instead, working with 10 mg of substrate, and an enantioselectivity of 96% ee was attained (Table 2, entry 11). To further assess the scope of this reduction, we next turned our attention to a set of indanone derivatives **1k–1o** having methyl, methoxy, or halogen substituents on the benzene ring (Table 2, entries 11–15). The corresponding alcohols **2k–2o** were uniformly obtained with quantitative yields and high levels of enantioinduction (ee >99%). Tetralone derivatives effected efficient catalytic enzymatic reductions under aqueous conditions providing the same results in terms of yield and enantioselectivity (Table 2, entries 16–17). To broaden the scope of the reaction, a linear compound **1r** was subjected to this biocatalytic process. The β -ketosulfone **1r** furnished the alcohol **2r** in quantitative yield and 98% ee (Table 2, entry 18).

Pleasingly, the reaction could be run at a substrate concentration of 2.47 M (500 mg) in the presence of the same amounts of enzyme (5 mg, 2.5% w/w) and NAD⁺ (0.4 mg, 0.02% mol) in 950 μ L of sodium phosphate buffer solution. The *i*PrOH quantity was finely adjusted to 40% vol (700 μ L, 3.7 equivalents) to ensure substrate solubility and a sufficient excess to complete the reaction without affecting the activity of the enzyme. In these conditions, substrate **1g** was quantitatively reduced into alcohol **2g** in similar yields (quant.) and enantioselectivity (ee >99%) as stated on smaller scale attesting to the effectiveness and usefulness of the enzymatic reduction.

When comparing the present results of enzymatic reductions of compounds **2a–2j** and **2r** with those using transition metal-based catalytic asymmetric transfer hydrogenation (ATH),^[19] the following conclusions can be drawn (Figure 3). In the case of oxygen and sulfur-containing heterocycles such as **2a–2c**, and **2h–i**, comparable results were obtained with enantioselectivities up to 99%. Slightly better enantioinductions were observed with **2e–2h** using biocatalysis (>99% ee vs 96% ee with a Rh complex). Equal enantiomeric excess was attained regarding sulfone containing compound **2r**. A significantly better ee value was obtained for **2d** by enzymatic reduction compared to Rh-catalyzed ATH (>99% ee vs 75% ee)^[19] and this is thus the method of choice for the reduction of compound **1d**.

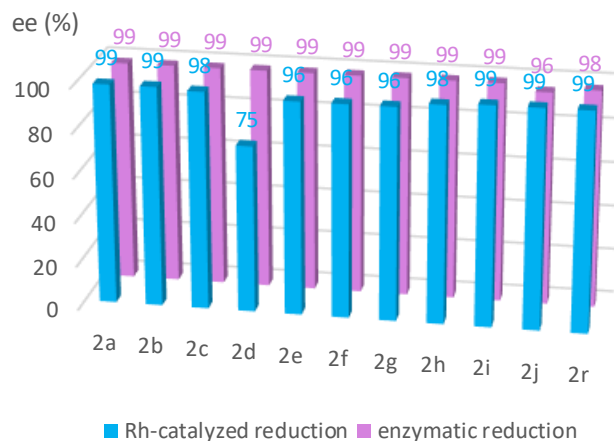


Figure 3. Enzymatic reduction vs metal-catalyzed asymmetric transfer hydrogenation.

In conclusion, we have developed an unexplored efficient enzymatic reduction to access a variety of diversely functionalized *gem*-difluorinated alcohols. The reduction proceeds under aqueous conditions without sacrificing reactivity and allows to provide a range of small fluorinated molecules including enantioenriched β -hydroxy *gem*-difluorinated heterocyclic and an acyclic derivative under mild conditions at room temperature. Upon subjecting *gem*-difluorinated ketones to the enzymatic reduction, quantitative yields and enantioselectivities up to >99% were obtained. Moreover, in developing a biocatalytic approach, one important consideration is the feasibility of accessing chiral fluorinated molecules under sustainable reaction conditions. Finally, upon performing a comparative study between enzymatic reduction and metal-catalyzed asymmetric transfer hydrogenation, we clearly demonstrated the efficiency of the enzyme collections provided by Protéus by SEQENS (Sequenzym[®] kits) and the complementarity of the two methods. Not only does the successful scale-up of this enzymatic transformation demonstrate its usefulness and practical potential but it also paves the way for a future industrial process development.

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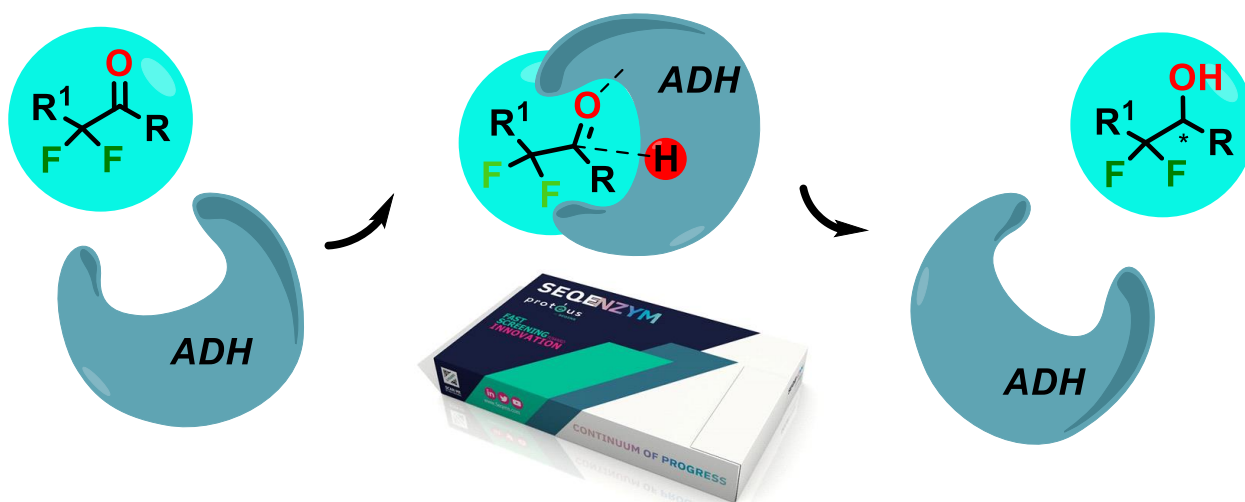
Experimental Section

General procedure for the enzymatic reduction

In a 1 mL vial equipped with a stirring bar, was added a solution of NAD(P)⁺ cofactor (0.4 mg) in 950 μ L of a freshly prepared 100 mM sodium phosphate buffer solution at pH 7. Then the enzyme E4408 (5 mg) was added to the cofactor solution and homogenized with a Pasteur pipette. A solution of ketone **1** (20 mg) in 200 μ L of *i*PrOH was prepared (heating can be necessary to solubilize the starting material) and was added to the enzyme/cofactor solution. The vial was sealed with a blue cap and stirred at 500 rpm and 30 °C during the required time in an oil bath. After checking the completion of the reaction by TLC, the reaction mixture was diluted in 1 mL of AcOEt. The aqueous phase was extracted with AcOEt (3 x 2 mL) and filtered through a pad of silica gel to obtain the pure product. The enantiomeric excess was measured by SFC or HPLC on a chiral stationary phase.

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