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Biocatalytic Approach to Chiral β-Nitroalcohols by Enantioselective Alcohol Dehydrogenase-Mediated Reduction of α-Nitroketones

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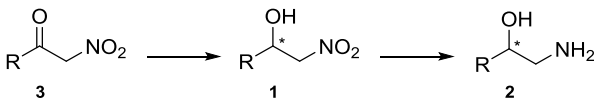
Abstract: Chiral β-nitroalcohols are important building blocks in organic chemistry. The synthetic approach that is based on the enzyme-mediated reduction of α-nitroketones has been scarcely considered. In this work, the use of commercial alcohol dehydrogenases (ADHs) for the reduction of aromatic and aliphatic nitroketones is investigated. High conversions and enantioselectivities can be achieved with two specific ADHs, affording either the (*S*) or (*R*)-enantiomer of the corresponding nitroalcohols. The reaction conditions are carefully tuned to preserve the stability of the reduced product, and to avoid the hydrolytic degradation of the starting substrate. The further manipulation of the enantioenriched nitroalcohols into Boc-protected amminoalcohols is also described.

Keywords: nitroketone; reduction; alcohol-dehydrogenase; enantioselectivity

1. Introduction

Chiral β-nitroalcohols **1** (Scheme 1) are relevant synthetic targets in organic chemistry. They are employed as key intermediates for the preparation of a wide range of biologically active natural products and active pharmaceutical ingredients [1–5], especially because they can be readily converted into chiral β-aminoalcohols **2** by reduction of the nitro moiety.



Scheme 1. Synthesis of β-amminoalcohols **2** through β-nitroalcohols **1** as intermediates

The most common approach to compounds **1** is represented by the enantioselective Henry (nitroaldol) reaction between aldehydes and nitromethane, which is catalysed by metal complexes or organocatalysts [1,6–16].

During the past decade, the search for greener and more sustainable synthetic procedures has promoted the investigation of biocatalysed strategies for the synthesis of enantiopure β-nitroalcohols [17]. Several examples of kinetic resolution of racemic compounds **1** catalysed by hydrolases have been reported in the literature [7]. It has also been discovered that some hydroxynitrile

lyases (HNLs) are able to promote the enantioselective addition of nitromethane to aldehydes, such as the (*S*)-selective HNLs from *Hevea brasiliensis* and from *Manihot esculenta* [18–20], and the (*R*)-selective HNLs from *Arabidopsis thaliana* [21], *Acidobacterium capsulatum,* and *Granulicella tundricula* [22]. These reactions are generally characterized by long reaction times, and strong substrate dependence.

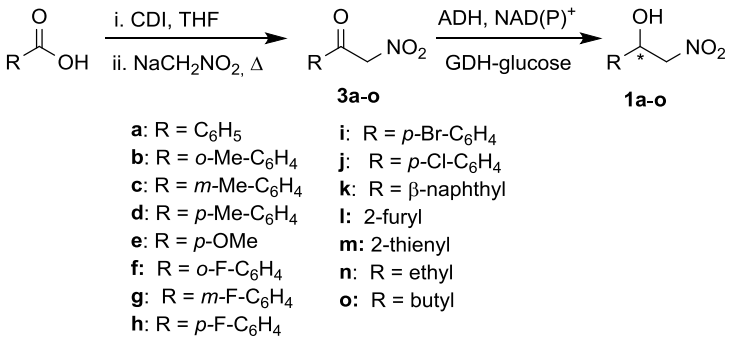
Another possible enzymatic approach, which has received scarce consideration until now, is represented by the bioreduction of α-nitroketones **3**. Only a few papers on this topic are present in the literature. In 1987 [23], the baker's yeast reduction of 3-methyl-3-nitro-2-butanone to the (*S*)-enantiomer of corresponding alcohol (enantiomeric excess = ee > 96%) in 57% yield was described. A few years later, Moran et al. [24] investigated the reduction of α-nitroacetophenone (**3a**. R = Ph) in fermenting baker's yeast. Only 6% of nitroalcohol **1a** (R = Ph) could be isolated, with benzoic acid being the main product of the biotransformation (27%). According to the authors, the formation of benzoic acid was due to the retro-Henry degradation of nitroalcohol **1a** to benzaldehyde, followed by oxidation. In 2008, Kroutil et al. [25] reported on the conversion of 1-nitro-3-phenylpropan-2-one and 1-nitro-2-octanone into the enantiopure (*S*)-nitroalcohols in 47% and 75% yield, respectively, by using the lyophilized cells of *Comomonas testoteroni.* Recently [26], the whole cells of *Candida parapsilosis* ATCC 7330 were employed to catalyse the enantioselective reduction of some aliphatic derivatives **3** (only R = alkyl) in water with ethanol as a cosolvent, at room temperature, and in 4 h reaction time (conversion yields 54–76%, ee = 8.2–81%). The formation of the (*R*) or (*S*) enantiomer of the corresponding nitroalcohol depended upon the nature of the R group.

The scarcity of experimental data on the bioreduction of α-nitroketones, especially for aromatic derivatives, and the current need for biocatalysed synthesis of chiral building blocks for pharmaceutical applications [27–31] led us to investigate the use of commercial alcohol dehydrogenases for the enantioselective reduction of aryl and alkyl α-nitroketones **3** in controlled reaction conditions. We also studied the further manipulation of specific nitroalcohols **1** to prepare aminoalcohols **2**, which have been already employed as key intermediates for the synthesis of active pharmaceutical ingredients, such as levamisole and (*R*)-tembamide.

2. Results and Discussion

2.1. Synthesis of Nitroketones 3 and Biocatalysed Reduction to Derivatives 1

Nitroketones **3a**–**o** (Scheme 2) were synthesized according to the literature by derivatization of the corresponding carboxylic acids with carbonyldiimidazole, followed by reaction with the sodium salt of nitromethane, which was obtained in turn by deprotonation of nitromethane with NaH [32].



Scheme 2. Synthesis and biocatalysed reduction of nitroketones **3a**–**o**.

Before starting the alcohol dehydrogenases (ADH) screening, the stability of derivatives **1** was investigated in buffer solutions at pH = 5, 7, and 9 for 4–18 h at 25 °C, using compound **1a** as a model and DMSO as a co-solvent. As expected, nitroalcohol **1a** resulted to be unstable towards retro-Henry reaction in basic and neutral medium: conversion into benzaldehyde was complete at pH = 7 and 9

after 18 h. At pH = 5 no benzaldehyde was observed. Thus, pH = 5 was selected for the investigation of the biocatalysed reduction of compound **3a**, using a panel of 18 commercial alcohol dehydrogenases (from Evoxx). The catalytic NADPH or NADH cofactor was recycled with glucose dehydrogenase (GDH from *Bacillus megaterium*), and glucose was employed as a sacrificial co-substrate. The reactions were performed in acetate buffer solution (pH = 5) with 1% DMSO, monitored by TLC, and usually stopped after 4–5 h. The results of the screening experiments are collected in Table S1. Conversion were evaluated by ^{1}H NMR spectroscopy and the enantiomeric excess values of the reduced products were determined by HPLC analysis on a chiral stationary phase. GC-analysis could not be used because nitroalcohol **1a** undergoes partial thermal degradation to benzaldehyde.

During this screening, benzaldehyde was never detected in the final reaction mixture, while the formation of benzoic acid was observed in a variable amount: from 4–6% in the most effective reductions of **3a** with ADH270 and 440, to nearly 30% in those reactions in which no nitroalcohol was formed. In order to explain the formation of benzoic acid, the stability of compound **3a** was investigated in buffer solution (pH = 5), in the presence of 1% DMSO, GDH, NAD(P)$^+$, without adding the ADH, for 4 and 18 h at 25 °C. Partial degradation (35%) to the carboxylic acid was observed after 4 h, while the complete conversion into benzoic acid was achieved after 18 h. A search in the literature showed that Pearson et al. [33] had described the hydrolytic cleavage of nitroketone **3a** to the corresponding carboxylic acid in dioxane-water solution and the possibility to suppress this side-reaction only in strong mineral acid solution. In the evaluation of the molar percentages of the reduced product **1a** reported in Table S1, as calculated by ^{1}H NMR analysis of the final mixture, the formation of the carboxylic acid was taken into account. The integrals of the following well-separated signals were employed: (i) the doublet of doublets of the CH-OH of **1a** (one hydrogen atom); (ii) the singlet of the CH_2 of **3a** (two hydrogen atoms); and, (iii) the doublet of the two aromatic hydrogen atoms adjacent to the COOH group of benzoic acid.

Only eight of the eighteen screened ADHs could catalyze the reduction of nitroketone **3a**. Prolonged reaction times did not improve the yield in the reduction product, instead promoted the extensive hydrolysis of unreacted starting **3a**. The ADHs giving the best results in terms of both conversion and enantioselectivity, i.e., ADH270, 440 and 441, were employed to investigate the reduction of the whole set of nitroketones **3b**–**o**. The results are reported in Figure 1 and Table S2. The absolute configuration of all the nitroalcohols **1a**–**o** could be established by a comparison of the corresponding HPLC analyses on chiral stationary phase with those reported in the literature in the same experimental conditions (See Supplementary Materials).

(*R*)-Nitroalcohols were invariably obtained in the presence of ADH440, while opposite enantioselectivity were observed with either ADH270 or ADH441. In the reduction of 2-furyl and 2-thienyl derivatives **3l** and **3m**, obtaining the (*S*)-nitroalcohol with ADH440 and the (*R*)-enantiomer with ADH270, and 441 does not represent an inversion of enantioselectivity with respect to the reductions of the other substrates. It is a consequence of the fact that the priority order of the substituents around the stereogenic centre is different for the presence of the heteroaromatic ring. The only real inversions of configuration were observed in the reduction of **3f** (R = *o*-F-C_6H_4) and **3n** (R = ethyl) with ADH441 and 270, respectively, affording the corresponding (*R*)-enantiomers with ee = 43 and 80%.

The best results were achieved while using ADH440 as a catalyst (Figure 1). This enzyme promoted the conversion of nitroketones **3** into the (*R*)-enantiomer of nitroalcohols **1** with high yields (c = 79–99%) and very good ee values in the range 92–99% for most of the substrates. Enantioselectivity that was slightly lower 90% was observed in the quantitative reduction of **3c** (R = *m*-Me-C_6H_4, ee = 84%) and **3l** (R = 2-furyl, ee = 71%). Only in the case of ethyl derivative **3n**, the corresponding reduced product was obtained in racemic form.

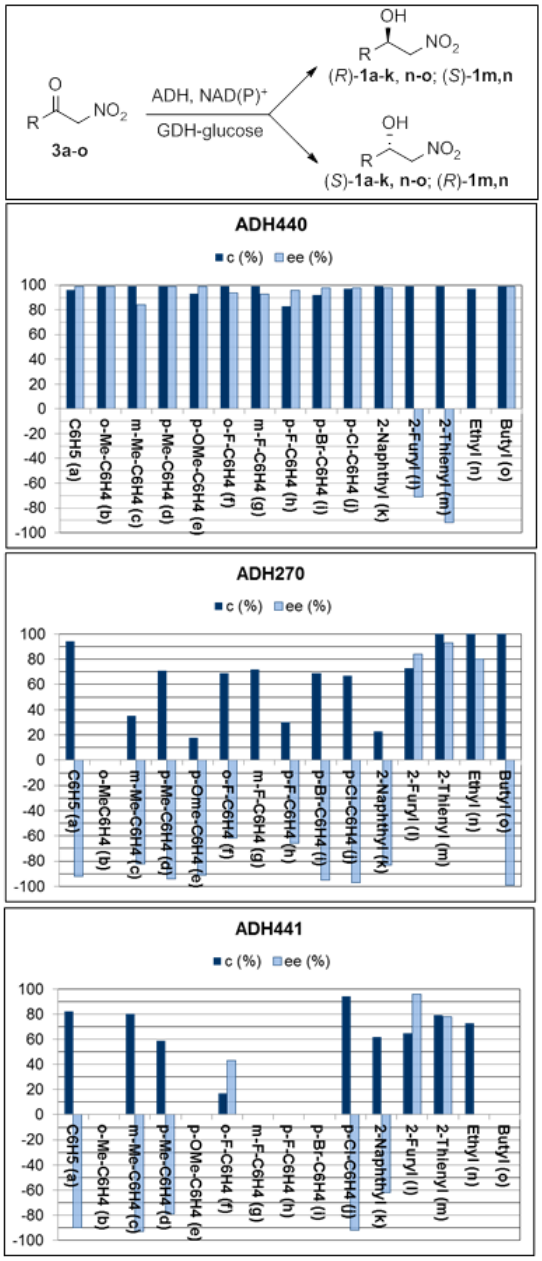


Figure 1. Alcohol dehydrogenases (ADH)-mediated reduction of nitroketones **3a**–**o** to nitroalcohols **1a**–**o** (preliminary screening). For graphic reasons the ee values of (*R*)-enantiomers are represented as positive values, those of (*S*)-enantiomers are given as negative: 5 mM substrate, 16 mM glucose, ADH, glucose dehydrogenase (GDH), $NAD(P)^+$, 1% DMSO, acetate buffer pH 5.0, 25 °C, 4–5 h; conversion (c, %) calculated by ^{1}H NMR spectroscopy as molar percentage of the nitroalcohol **1** in the final reaction mixture after 4–5 h, taking into account the unreacted nitroketone **3**, and the carboxylic acid obtained upon nitroketone hydrolysis; enantiomeric excess (ee, %) calculated on the basis of HPLC analysis on a chiral stationary phase.

ADH270 gave the (*S*)-enantiomer of the reduced product in all the bioreductions, with the exception of the reaction of compound **3n** (Figure 1, R = ethyl), affording (*R*)-**1n** (ee = 80%). The highest ee values (91–99%) were obtained in the transformation of *para*-substituted nitroketones **3d** (R = *p*-Me-C_6H_4, ee = 94%), **3e** (R = *p*-OMe-C_6H_4, ee = 91%), **3i** (R = *p*-Br-C_6H_4, ee = 95%), **3j** (R = *p*-Cl-C_6H_4, ee = 97%), and derivatives **3a** (R = phenyl, ee = 92%), **3m** (R= 2-thienyl, ee = 93%), and **3o** (R = butyl, ee = 99%). Enantioselectivity in the range 80–84% was achieved in the reduction of

compounds **3c** (R = *m*-Me-C_6H_4, ee = 82%), **3k** (R = 2-naphthyl, ee = 83%), **3l** (R = 2-furyl, ee = 84%), and **3n** (R = ethyl, ee = 80%), while modest ee values could be obtained with fluoro derivatives **3f** (R = *o*-F-C_6H_4, ee = 62%), and **3h** (R = *p*-F-C_6H_4, ee = 66%). *m*-Fluoro nitroketone **3g** was converted into a racemic nitroalcohol. Only substrate **3b** (R = *o*-Me-C_6H_4) was recovered unreacted.

When ADH441 was employed as a catalyst (Figure 1), the relevant results were achieved in the reduction of **3a** (R = Ph), **3c** (R = *m*-Me-C_6H_4), **3j** (R = *p*-Cl-C_6H_4), and **3l** (R = 2-furyl), affording the corresponding nitroalcohol with high enantiomeric purity (ee = 90, 93, 92 and 96%, respectively).

The results of this screening clearly show that ADH270 and 440 are the most effective catalysts for the preparation of both the enantiomers of nitroalcohols **1**.

2.2. Bioreductions of Nitroketones in Biphasic Medium

In order to avoid the drawback of nitroketone hydrolysis, the use of a biphasic medium (buffer and organic solvent) was evaluated. No benzoic acid was observed when compound **3a** was stirred in toluene/buffer or EtOAc/buffer mixtures for 24 h in the presence of GDH and $NAD(P)^+$ without adding the ADH. In the presence of ADH440 and ADH270 as catalysts, the reductions proceeded affording the results that are reported in Table 1. Toluene resulted to be the solvent of choice, preserving nitroketone **3a** from hydrolysis, still maintaining the activity of the ADH.

Table 1. ADH-mediated reduction of nitroketone **3a** to nitroalcohol **1a** in biphasic system [a].

ADH [1]	Organic Solvent	Conversion [2] (%)	Ee [3] (%)
270	AcOEt	-	-
440	AcOEt	68	98 (*R*)
270	toluene	88	95 (*S*)
440	toluene	99	97 (*R*)

[1] Total volume 4 mL (organic solvent/water 1/1), 6 mM substrate, 20 mM glucose, ADH (2 mg), GDH (1 mg), $NAD(P)^+$ (0.25 mM), acetate buffer pH 5.0, 25 °C, 24 h; [2] conversion calculated on the basis of the ^{1}H NMR spectrum of the crude mixture after 24 h; [3] enantiomeric excess calculated on the basis of HPLC analysis on a chiral stationary phase.

The ADH-mediated reduction of model nitroketone **3a** was also investigated in 1:1 toluene-water (buffer pH = 5) at 25 °C with ADH270 and 440 in order to increase both substrate loading (mg/mL) and substrate to enzyme ratio (mg/mg). The corresponding conversions, determined after 24 h reaction time by ^{1}H NMR spectroscopy, are reported in Table 2.

Table 2. Effect of substrate concentration and substrate/enzyme ratio on conversion for the ADH-mediated reduction of **3a**.

ADH [1]	[Substrate](mg/mL)	Substrate/Enzyme(mg/mg)	Conversion [2](%)
440	1	2.0	99
	1	8.0	94
	2	2.0	95
	2	8.0	91
	3	8.0	94
	3	24.0	80
270	1	2.0	88
	1	3.0	67
	2	2.0	84
	2	3.0	74
	3	3.0	73
	3	4.0	58

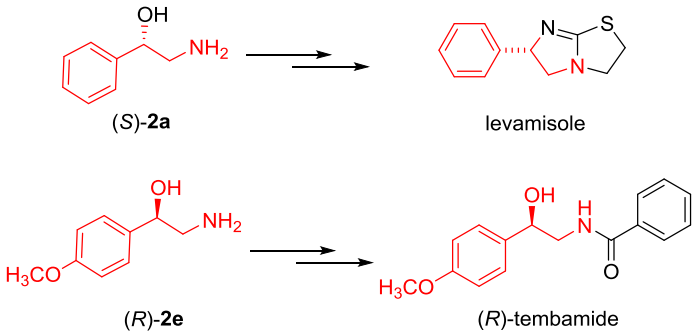
[1] Total volume 4 mL (organic solvent/water 1/1), substrate, glucose (3.2 eq), ADH, GDH, $NAD(P)^+$ (0.04 eq), acetate buffer pH 5.0, 25 °C, 24 h; [2] conversion calculated on the basis of the ^{1}H NMR spectrum of the crude mixture after 24 h.

ADH440 was found to be very effective in promoting the reduction of substrate **3a:** in batch conditions, with a substrate concentration of 3 mg/mL, conversion remained still satisfactory (80%) when the enzyme concentration was decreased from 0.38 mg/mL (substrate/enzyme = 8) to 0.12 mg/mL (substrate/enzyme = 24).

ADH 270 showed less efficiency than ADH440 in these bioreductions. When substrate loading was increased to 3 mg/mL the use of 1 mg/mL enzyme (substrate/enzyme = 3) afforded 73% conversion, while a further decrease of enzyme concentration to 0.75 mg/mL (substrate/enzyme = 4) led only to 58% of reduced product.

2.3. Synthesis of Boc-protected β-Aminoalcohols **2**

The conversion of β-nitroalcohols **1** into β-aminoalcohols **2** was investigated, in order to establish the synthetic potential of the ADH-mediated reduction of nitroketones **3** and highlight its value within organic chemistry procedures. Compounds **1a** and **1e** were employed as model substrates, since the corresponding amino derivatives (*S*)-**2a** and (*R*)-**2e** are the key intermediates in the synthesis of active pharmaceutical ingredients, such as levamisole and (*R*)-tembamide (Scheme 3).

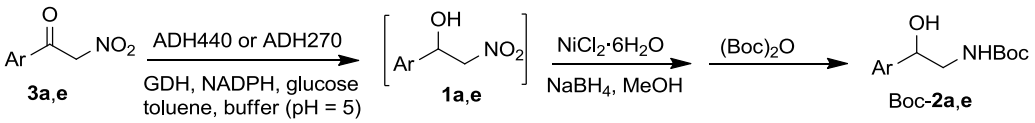


Scheme 3. Active pharmaceutical ingredients prepared starting from amino alcohols (*S*)-**2a** and (*R*)-**2e**.

Levamisole, which is the (*S*)-enantiomer of tetramisole, is a broad spectrum anthelmintic [34], which has found wide application in the treatment of worm infestations and in the elimination of intestinal parasites in both humans and animals. It is also one of the nonspecific immunomodulating agents that are used in clinical practice [35–37]. The known synthetic asymmetric approaches are based on the use of optically active phenylethylenediamine [38–41] or amino alcohol (*S*)-**2a** as intermediates [42].

(*R*)-(−)-Tembamide is a naturally occurring β-hydroxyamide isolated from various members of the Rutaceae family. This compound has been reported to have insecticide and adrenaline-like activity. Extracts of *Aegle marmelos*, containing tembamide, have been used in the Indian traditional medicine as a control for hypoglycemia [43]. Most of the enantioselective procedures to (*R*)-tembamide involve the use of the corresponding amino alcohol (*R*)-**2e** as a key building block [44].

The nitro moiety of model compounds (*S*)-**1a** and (*R*)-**1e** was converted into the corresponding amino functionality by reaction with $NiCl_2 \cdot 6H_2O$ and $NaBH_4$ (Scheme 4), followed by treatment with $(Boc)_2O$, in order to facilitate the isolation of the aminoalcohol from the reaction mixture. The reaction was fast and is characterized by complete conversion. The Boc derivatives could be recovered as solid compounds, and easily purified by crystallisation. The procedure was carried out directly in the reaction medium of the biocatalysed reaction, after removal of the aqueous phase, avoiding the isolation of the intermediate nitroalcohols, in order to achieve a one-pot chemo-catalysed conversion of nitroketones **3a** and **3e** into Boc-protected derivatives (*S*)-**2a** and (*R*)-**2e**, in 57 and 63% isolation yields.



Scheme 4. Synthesis of Boc-protected derivatives **2**.

3. Materials and Methods

3.1. Sources of Enzymes

GDH from *Bacillus megaterium* DSM509 (DSM, Heerlen, the Netherlands) was overexpressed in *E. coli* BL21 (DE3) strains harbouring the plasmid pKTS-GDH prepared according to standard molecular biology techniques. The enzyme was produced and purified, as described in the Supplementary Materials.

The complete set of ADHs was purchased from Evoxx (Monheim am Rhein, Germany).

3.2. General Procedure for the ADH-Mediated Reduction of α-Nitroketones **1a**–**o** *(Screening)*

A solution of the substrate in DMSO (50 μL, 500 mM) was added to an acetate buffer solution (5 mL, 50 mM, pH 5.0) containing glucose (80 μmol), $NADP^+$ (1 μmol, Sigma-Aldrich, Milan, Italy) or NAD^+ (1 μmol, Sigma-Aldrich, Milan, Italy) (according to the ADH preference), GDH (1.5 mg), and the required ADH (3 mg, Evoxx, Monheim am Rhein, Germany). The mixture was incubated for 4–5 h in an orbital shaker (150 rpm, 30 °C). The solution was extracted with EtOAc (2 × 1 mL, Sigma-Aldrich, Milan, Italy), centrifuging after each extraction (15,000 *g*, 1.5 min). The combined organic solutions were dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was submitted to ^1H NMR analysis (Bruker, Milan, Italy) to determine conversion. Two replicates were performed for each biotransformation: no significant differences (less than 5%) were observed for conversion and enantiomeric excess values.

The enantiomeric excess values of each nitroalcohol was determined by HPLC analysis (Agilent, Cernusco sul Naviglio, Italy) on a chiral stationary phase (See Supplementary Materials). The comparison of these HPLC analyses with those that were reported in the literature in the same experimental conditions (See Supplementary Materials) allowed for the absolute configuration of nitroalcohols **1a**–**o** to be established.

3.3. General Procedure for the Reduction of Nitroketone **3a** *in a Biphasic System Mediated by ADH440 and ADH270.*

A solution of nitroketone **3a** (4 mg, 25 μmol) in toluene (2 mL) was mixed with an acetate buffer solution (2 mL, 50 mM, pH = 5), containing glucose (80 μmol), $NADP^+$ (1 μmol), GDH (1 mg), and the required ADH (2 mg). The mixture was incubated for 24 h in an orbital shaker (150 rpm, 30 °C). The mixture was extracted with EtOAc (2 × 1 mL), centrifuging after each extraction (15,000 *g*, 1.5 min). The combined organic solutions were dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was submitted to ^1H NMR analysis to determine conversion. Two replicates were performed for each biotransformation: no significant differences (less than 5%) were observed for the conversion and enantiomeric excess values.

The same procedure was employed to investigate the effect on conversion due to substrate loading and substrate to enzyme ratio, by changing the amount of nitroketone and ADH.

3.4. General Procedure for the Conversion α-Nitroketones **3a** *and* **3e** *into Boc Protected Amino Alcohols* **2a** *and* **2e**

The enantioselective reduction of the nitroketone was performed with the required ADH on 100 mg of nitroketone (20 mL of toluene, 20 mL of buffer pH = 5, 15 mg of ADH440 or 35 mg of ADH270, 10 mg $NADP^+$, 7.5 or 18 mg GDH, 150 mg glucose), following the procedure already described in the previous paragraph for biotransformations in biphasic medium. After 24 h, the aqueous phase was

removed, methanol was added (0.5 mL), followed by the cautious addition of $NiCl_2 \cdot 6H_2O$ (1 eq) and $NaBH_4$ (3 eq) under vigorous stirring. After 30 min, $(Boc)_2O$ (1.2 eq) was added. The mixture was stirred for 30 min, filtered through a celite pad, and extracted with EtOAc. The organic layers were dried over anhydrous Na_2SO_4, and the residue was purified by crystallization from hexane–EtOAc.

3.4.1. (*S*)-*Tert*-butyl (2-hydroxy-2-phenylethyl) carbamate ((*S*)-**2a**)

From compound **3a** (100 mg, 0.61 mmol), using ADH270, derivative (*S*)-**2a** was obtained (83 mg, 57%): ee (HPLC) = 92%, $[\alpha]_D$ = +46.8 (*c* 0.85, $CHCl_3$) [lit. ref. [45] $[\alpha]_D$ = +45.1. (*c* 0.6, $CHCl_3$) for (*S*)-1a with ee = 93%]; ^{1}H NMR ($CDCl_3$, 400 MHz) [39]: δ = 7.40–7.27 (m, 5H, ArH), 4.92 (br s, 1H, *NH*), 4.83 (m, 1H, *CH*OH), 3.48 (m, 1H, *CH*N), 3.26 (m, 1H, *CH*N), 3.01 (br s, 1H, OH), 1.45 (s, 9H, $(CH_3)_3$C); ^{13}C NMR ($CDCl_3$, 100.6 MHz) [45]: δ = 157.1, 142.0, 128.6, 127.9, 126.0, 80.0, 74.0, 48.5, 28.5; GC-MS (EI) t_r= 21.5 min *m*/*z* (%) = 181 (M^+-56, 14), 107 (100), 79 (47), 57 (100).

HPLC analysis [45]: Chiralcel OD, 95/5 hexane/*i*-PrOH, 0.6 mL/min, 215 nm, (*R*)-**2a** t_r = 19.1 min, (*S*)-**2a** t_r = 23.4 min.

3.4.2. (*R*)-*Tert*-butyl (2-hydroxy-2-(4-methoxyphenyl)ethyl) carbamate ((*R*)-**2e**)

From compound **3e** (100 mg, 0.51mmol), using ADH440, derivative (*R*)-**2e** was obtained (86.3 g, 63%): ee (HPLC) = 96%, $[\alpha]_D$ = −37.7 (c 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) [46]: δ = 7.28 (d, *J* = 8.7 Hz, 2H, ArH), 6.88 (d, *J* = 8.7 Hz, 2H, ArH), 4.95 (br s, 1H, *NH*), 4.76 (m, 1H, *CH*OH), 3.80 (s, 3H, OCH_3), 3.43 (m, 1H, *CH*N), 3.23 (m, 1H, *CH*N), 3.00 (br s, 1H, OH), 1.44 (s, 9H, $(CH_3)_3$C); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 159.4, 157.0, 134.1, 127.2, 114.1, 79.9, 73.2, 55.4, 48.5, 28.5; GC-MS (EI) t_r= 23.8 min m/z (%) = 267 (M^+, 0.5), 211 (5), 137 (100), 109 (15), 57 (18).

HPLC analysis: Chiralcel OD, 95/5 hexane/*i*-PrOH, 0.6 mL/min, 215 nm, (*R*)-**2a** t_r = 26.9 min, (*S*)-**2a** t_r = 34.9 min.

4. Conclusions

The results that are reported in this work show that the biocatalytic reduction of α-nitroketones **3** mediated by ADHs is a convenient and useful procedure for the synthesis of both the enantiomers of the corresponding β-nitroalcohols **1** with high enantiomeric purity. In particular, for the first time the reduction of aryl and heteroaryl α-nitroketones (R = aryl or heteroaryl) has been successfully achieved by enzymatic catalysis, enlarging the known methods for the reduction of these compounds limited up to now to the asymmetric transfer hydrogenation in the presence of ruthenium [47], and iridium [48] chiral complexes, with formic acid as a reductant.

The bioreduction is performed under mild conditions (ambient temperature and pressure), with low energy consumption, at the expense of glucose, which is employed as a sacrificial substrate for the enzymatic regeneration of the cofactor. The enzymes catalyzing this transformation with either (*R*)- and (*S*)-selectivity are commercially available, and they can be manipulated easily and safely. The use of a biphasic reaction medium with toluene as an organic solvent does not inhibit the activity of the selected ADHs, helps in preserving the starting substrate from hydrolytic degradation, and it improves work-up procedures. The further manipulation of nitroketones into aminoalcohols was carried out without isolation of the nitroalcohol intermediate, thus telescoping the synthetic sequence.

Future work will be devoted to increase the productivity of the reaction, for example, by immobilizing the most suitable ADHs on solid supports and performing the reaction in flow conditions.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4344/8/8/308/s1.

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