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(*R*)-1-Arylethanols from aryl iodides through a two-step one-pot enantioselective chemoenzymatic process

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ABSTRACT

(*R*)-1-Arylethanols have been prepared in high to excellent overall yields through a two-step one-pot process that involves the palladium-catalyzed conversion of aryl iodides into the corresponding acetophenones, in the presence of acetic anhydride, EtN(*i*-Pr)$_2$, LiCl, and Pd$_2$(dba)$_3$ followed by an enantioselective reduction step catalyzed by the alcohol dehydrogenase enzyme from *Lactobacillus* brevis.

1. Introduction

Chiral secondary alcoholic fragments are a common motif of many biologically active compounds [1]. Chiral secondary alcohols are also key intermediates [2] for the synthesis of various pharmaceutical products (the need for enantiomerically pure drugs has dramatically increased in recent years). Because of this, a large number of methods have been developed for their preparation from ketones via enantioselective reduction using conventional reducing agents [3], transition metal- [4], and enzyme-catalyzed [5] processes. Chemoenzymatic dynamic kinetic resolution has also been used [6]. All these procedures are based on the conversion of the preformed carbonyl-containing precursor into the desired chiral products. Therefore, the development of an alternative one-pot process involving the introduction of the carbonyl functionality into readily available substrates followed by a stereoselective enzymatic reduction step appeared to us particularly attractive. Based on the great versatility of palladium in C–C bond forming reactions and the ability of alcohol dehydrogenases to perform stereoselective reduction reactions of ketones, we focused on a chemoenzymatic process that entails a palladium-catalyzed step (the formation of acetophenones **2** from aryl iodides **1**) followed by an enzyme-catalyzed enantioselective reduction step (the generation of optically pure 1-arylethanols **3** from **2**) (Scheme 1).

Efficient and industrially feasible one-pot chemoenzymatic methodologies, circumventing intermediate purification and isolation steps, would represent a promising asymmetric route to chiral 1-arylethanols due to the significant advantages offered in terms of efficiency and economy as well as environmental aspects over stepwise processes. However, one-pot syntheses based on transition metal and enzyme catalysts can be extremely challenging, particularly when they involve a C–C bond forming step. Indeed, organometallic reagents are commonly used in these cases to generate the new C–C bonds and metals or reagents can inhibit the enzyme. In addition, although it has been shown that non-aqueous media can be used with enzymes, which allows for the solubilization of hydrophobic substrates, water/organic solvent mixtures need to be optimized. We recently reported [7] that a variety of acetophenones can be prepared in good to excellent yields from neutral, electron-rich, and electron-poor aryl iodides under monoxide free conditions, without using organometallic reagents as carbon donors, in the presence of acetic anhydride, EtN(*i*-Pr)$_2$ (acting as a reducing agent), LiCl, and catalytic amounts of Pd$_2$(dba)$_3$. Consequently, these conditions appeared to us particularly suited for developing a chemoenzymatic approach to chiral 1-arylethanols. Herein we present the results of a study in which the broad range, (R)-selective alcohol dehydrogenase from *Lactobacillus brevis* has been successfully coupled to the Pd catalyzed step.

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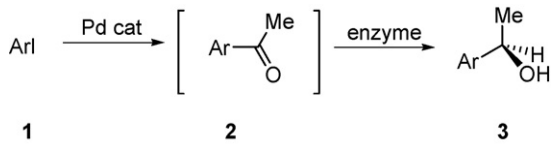


Scheme 1.

2. Materials and methods

2.1. Chemicals and enzyme

All reagents were purchased by Sigma–Aldrich Co. The gene for the R-specific alcohol dehydrogenase from *Lactobacillus brevis* (adh) was amplified using the following oligonucleotides: ADHNterm 5′-GAGGTCGTTCATATGTCAAACCGG-3′ and ADHCterm 5′-CGTTCCTTTTTGGATCCAAGTTATTGAGC-3′, using the genomic DNA as a template [8]. The amplified gene was then cloned between NdeI and BamHI restriction sites of the pET22b expression vector (Novagen, Madison, WI, USA) and sequenced. *Escherichia coli* BL21 (DE3) star cells (Invitrogen) transformed with pET22b-adh plasmid were grown overnight in LB medium supplemented with 100 mg/l ampicillin at 25 °C. The protein was expressed for 7 h at 30 °C, upon induction with 1 mM isopropyl-1-thio-β-D-galactopyranoside. The cells were harvested by centrifugation, pelletted, frozen overnight, then resuspended and sonicated in 10 mM phosphate buffer, pH 7.2, containing 1 mM phenylmethylsulfonyl fluoride (PMSF). The extract was centrifuged and the resultant supernatant was applied to a DEAE-sepharose column equilibrated with 10 mM phosphate buffer, pH 7.2. The column was washed with the same buffer and the recombinant protein was eluted with a linear gradient of sodium chloride from 0.0 to 0.5 M with a purity >75% as judged by densitometric analysis of SDS gel electrophoresis. The activity of the enzyme was determined photometrically at 340 nm in a mixture of 4 mM ethyl acetoacetate, 20 μM NADPH, in 100 mM sodium phosphate buffer pH 7.2. One unit of enzyme activity was defined as the amount of enzyme catalyzing the conversion of 1 μmol min^{-1} substrate at 25 °C under these conditions.

2.2. Synthesis of (R)-1-arylethanols

A Carousel Tube Reaction (Radley Discovery), equipped with a magnetic stirrer, was charged with different aryl iodides (**1a**, **1b**, **1c**, **1d**, **1e**; 0.5 mmol), Ac_2O (236 μl, 2.5 mmol), $Pd(OAc)_2$ (5.7 mg, 0.006 mmol), LiCl (106 mg, 2.5 mmol), and $EtN(i\text{-}Pr)_2$ (174 μl, 1.0 mmol) in 1 mL of anhydrous DMA. The reaction mixture was warmed at 100 °C and stirred at the same temperature for 5 h under argon. After cooling at room temperature, buffer phosphate (5 mL, 0.2 M, pH 7.2) was added and the mixture was warmed at 40 °C for 1 h. After that, phosphate buffer (24 mL, 0.2 M, pH 7.2), *i*-PrOH (3.6 mL), ADH-LB (1 mg, 69 U/mg, in 0.5 mL phosphate buffer 0.2 M pH 7.2), and $NADP^+$ (0.4 mL 10 mM) were added. The reaction mixture was stirred at room temperature for 3 h and subsequently extracted with AcOEt. After drying over magnesium sulphate, the organic phase was evaporated and the resulting crude product was purified by chromatography on silica gel eluting with a *n*-hexane/AcOEt 75/25 (v/v) mixture to give (*R*)-1-arylethanols.

2.3. Analytical procedures

Enantiomeric excess was determined by enantioselective HPLC using the following chiral stationary phase/eluent system: **3a**, Chiralpak AS-H/n-hexane-2-propanol 90/10; **3b**, Chiralcel OB-H/n-hexane-2-propanol 90/10; **3c**, Chiralpak IA/n-hexane-ethyl acetate-ethanol 95/5/0.5; **3d**, Chiralcel OJ-H/n-hexane-ethanol 95/5; **3e**, Chiralpak AS-H/n-hexane-2-propanol 50/50.

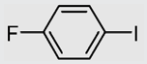
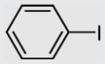
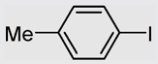
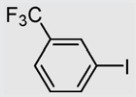
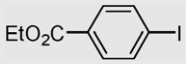
GC/MS analyses were performed on an Agilent 6850A gas chromatograph coupled to a 5973N quadrupole mass selective detector (Agilent Technologies, Palo Alto, CA, USA). Gas-chromatographic separations were carried out on an Agilent HP-5MS fused-silica capillary column (30 m, 0.25 mm I.D., film thickness, 0.25 μm). Injection mode: splitless at a temperature of 260 °C. Column temperature program: 70 °C (1 min) then to 280 °C at a rate of 10 °C/min and held for 15 min. The carrier gas was helium at a constant flow of 1.0 mL/min. The spectra were obtained in electron impact mode at 70 eV ionization energy and a mass range scan from *m*/*z* 30 to 500; ion source temperature, 280 °C; ion source vacuum 10^{-5} Torr.

3. Results and discussion

As enantiopure fluorinated compounds play a key role in medicinal chemistry [9], 4-fluoroiodobenzene [10] **1a** was selected as the model substrate. The two-step, one-pot process was carried out as follows. In the first step (the palladium-catalyzed acetylation), a commercial sample of **1a**, used directly without further purification, was subjected to acetic anhydride in the presence of $Pd_2(dba)_3$, $EtN(i\text{-}Pr)_2$, LiCl in DMA at 100 °C for 5 h. In the second step (the enzyme-catalyzed reduction), after cooling the reaction mixture to room temperature, a buffer phosphate solution was added and the mixture was warmed at 40 °C for 1 h to hydrolyze the acetic anhydride excess. Thereafter, additional phosphate buffer solution, *i*-PrOH, alcohol dehydrogenase enzyme from *Lactobacillus* brevis (LB-ADH), and $NADP^+$ were added and the resulting reaction mixture was stirred at room temperature. After a reaction time of 3 h, the desired product (*R*)-4-fluorophenylethan-1-ol was formed with a satisfactory overall yield of 86% and an enantiomeric excess of 98%. In situ conversion of $NADP^+$ to the required reduced form, NADPH, is catalyzed by the alcohol dehydrogenase enzyme from *Lactobacillus* brevis with, *i*-PrOH acting as the hydrogen transfer agent. *i*-PrOH excess makes the whole process irreversible, thus shifting the equilibrium towards the direction of the desired product. DMA was used in the palladium-catalyzed step instead of DMF [6] because it was found to limit the formation of benzoic acid byproducts most probably due to the decarbonylation of DMF [11]. For example, subjecting iodobenzene to the above conditions in DMF afforded benzoic acid in 25% yield along with the desired 1-arylethanol whereas no evidence of benzoic acid was attained when DMA was used.

LB-ADH activity towards ethyl acetoacetate was also essayed as described under methods in the presence of increasing amount of $Pd(OAc)_2$. As shown in Fig. 1, inhibitory effects of Pd were observed at metal concentrations higher than 2 mM, *i.e.* well above the catalytic concentrations used during the experiments (0.03 mM, after dilution with phosphate buffer). Having established that the enzymatic activity is not inhibited by the above conditions, other aryl iodides were next targeted for the chemoenzymatic process. As shown by the results summarized in Table 1, neutral, electron-rich and electron-poor aryl iodides can be accepted as a substrate by the alcohol dehydrogenase from *Lactobacillus* brevis and afforded the corresponding (*R*)-1-arylethanols in high to excellent overall yields and an enantiomeric excess ranging from 98 to >99%. The absolute configuration assignment was achieved by empiric analysis of circular dichroism (CD) properties of **3a–e**. The CD spectra of heptane solutions of commercially available (*R*)-(+)-phenylethanol enantiomer and **3a–d** exhibited a single positive Cotton effect at around 210 nm due to ^1L_a aromatic transition [12]. A shift at longer wavelength (225 nm) of diagnostic CD band for **3e** was observed.

Table 1
Preparation of (*R*)-benzylic alcohols **3** from aryl iodides **1** through a two-step one-pot palladium-catalyzed acetylation followed by LB-ADH-catalyzed reduction step.

Entry	Aryl iodide **1**		*t*(h)			Yield% of **3**[a]	ee (%)[b]	$[\alpha]_D^{20}$
			1st step	2nd step				
1		**1a**	5	4	**3a**	86 (80)	98	+42[c]
2		**1b**	40	3	**3b**	76	98	+50[d]
3		**1c**	8	3	**3c**	76 (70)	>99	+32[c]
4		**1d**	5.5	3	**3d**	92 (86)	>99	+53[d]
5		**1e**	7	3	**3e**	90 (85)	>99	+34[d]

[a] Yields are calculated by GC/MS analysis. Yields in parentheses refer to isolated products.
[b] The ee was determined by enantioselective HPLC.
[c] *c* = 0.2, MeOH.
[d] *c* = 0.1, MeOH.

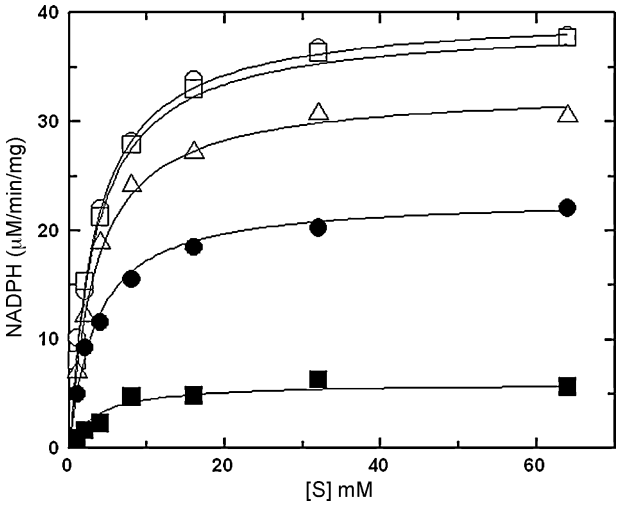


Fig. 1. Inhibition of *Lactobacillus brevis* ADH in the presence of $Pd(OAc)_2$. Initial velocities expressed as NADH consumption per minute per mg LB-ADH are plotted as a function of ethyl acetoacetate substrate (S) concentrations in the presence of varying amounts of $Pd(OAc)_2$: (empty circles) buffer; (empty squares) 1 mM; (empty triangles) 2 mM; (filled circles) 5 mM; (filled squares) 10 mM. Experimental conditions: 0.1 M phosphate buffer, pH 7.2, *T* = 25 °C, NADPH = 20 μM. Continuous lines represent the least squares fitting curves according to the simple Micaelis and Menten equation.

4. Conclusions

To sum up, we have shown that palladium and enzyme catalysis can be efficiently coupled to provide a two-step one-pot chemoenzymatic approach to chiral 1-arylethanols from aryl iodides. The process is simple and short, affords the desired products in high to excellent isolated yields with excellent enantiomeric excess and pave the way to a number of applications by coupling the versatility of palladium in C–C bond forming reactions to the appropriate enzyme catalyst.

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