Simple Biocatalytic Access to Enantiopure (*S*)-1-Heteroarylethanols Employing a Microbial Hydrogen Transfer Reaction

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Abstract: Lyophilised cells of various *Rhodococcus* spp. were employed in an efficient hydrogen transfer-like process for the asymmetric bioreduction of heteroaryl methyl ketones using 2-propanol as hydrogen donor. Besides the genus *Rhodococcus,* only *Mycoplana rubra* R14 showed a comparable stability towards elevated concentrations of the co-substrate 2-propanol. Among the organisms tested, *Rhodococcus ruber* DSM 44541 and DSM 43338 showed best activity and selectivity. With these strains, the reaction proceeded with high stereoselectivity (ee >99%) and predictable stereochemical outcome regardless of the nature of the heteroaromatic ring system. The reaction could be performed at the exceptional substrate concentration of up to 0.4 mol L^{-1} in an environmentally friendly aqueous-organic solvent mixture at room temperature and is easy to handle, thus providing a very practical tool to access enantiopure 1-heteroarylethanols.

Keywords: biotransformation; enzyme catalysis; 1-heteroarylethanol; hydrogen transfer; oxidoreductase

Introduction

Chiral 1-heteroaryl-1-alkanols are widely used as chiral ligands[1] and as building blocks for the synthesis of bioactive compounds.[2] The chiral heteroaryl alcohols are generally prepared by chemical or biochemical methods. Examples for chemical methods are (i) the asymmetric alkylation of aldehydes,[3] (ii) the asymmetric reduction of the corresponding ketones *via* hydrogenation,[4] transfer hydrogenation,[5] by using complex hydrides[6] or *via* hydrosilylation,[7] (iii) the oxidative kinetic resolution employing Sharpless' reagent[8] and (iv) the asymmetric hydroboration of styrene derivatives.[9] Examples for enzymatic methods are (i) the kinetic resolution of the *rac*-alcohol or the corresponding *rac*-esters by lipases,[10] (ii) the oxidative kinetic resolution of the *rac*-alcohol[11] or (iii) the asymmetric reduction employing whole (fermenting) cells or isolated enzymes,[11b,12] (iv) benzylic biohydroxylation of the corresponding arylalkane[12] and (v) microbial stereoinversion.[13]

Most of the biocatalytic methods show either high activity and low enantioselectivity or they give high enantiomeric excess but have low activity. All biocatalytic approaches are hampered by low substrate and therefore low product concentration and in the case of kinetic resolutions, the theoretical yield of each enantiomer is limited to 50%.

We recently reported the synthesis of chiral *m*- and *p*-substituted 1-phenylethanols *via* asymmetric biocatalytic hydrogen-transfer employing lyophilised cells of *Rhodococcus ruber* DSM 44541.[14,15] The high efficiency of this system is a result of the exceptional tolerance of the *sec*-alcohol dehydrogenase involved towards elevated concentrations of co-substrates used as hydrogen donor or acceptor. Thus, 2-propanol (up to 50% v/v) can be used as hydrogen donor for the reduction of ketones[16] and acetone (up to 25% v/v) serves as hydrogen acceptor for the oxidative kinetic resolution of *sec*-alcohols.[17] The high concentration of the water-miscible co-substrate not only drives the reaction into the desired oxidation or reduction direction, but also ensures sufficient reaction rates by acting as co-solvent for the solubilisation of lipophilic – and thus poorly water-soluble – substrates.

Results and Discussion

In order to extend this useful method to heteroaryl analogues, we initiated a screening for the biocatalytic reduction of various heteroaromatic ketones in the presence of 2-propanol as hydrogen donor at elevated concentrations (Scheme 1). The aim was (i) to identify organisms which reduce heteroaryl ketones and (ii)

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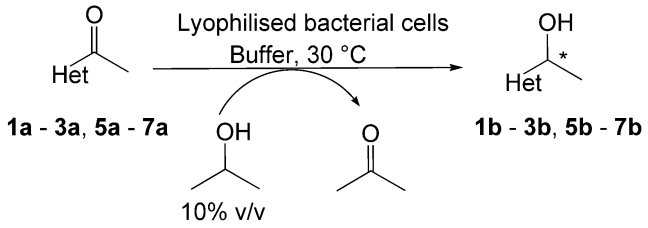
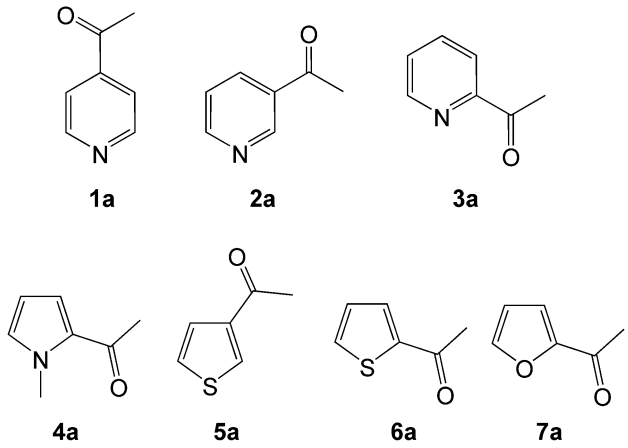


Scheme 1. Asymmetric reduction of heteroaromatic ketones via biocatalytic hydrogen transfer.

which tolerate 2-propanol as hydrogen donor, which would provide a simple and reliable access to chiral 1-heteroaryl-1-ethanol derivatives in high enantiomeric excess at high conversion. All screening experiments were performed at 10% v/v 2-propanol and 9 g L^{-1} substrate concentration employing lyophilised cells in phosphate buffer (50 mM, pH 7.5).

In a first approach, 20 organisms, used as lyophilised cell powder, were screened with 4-acetylpyridine **1a** using 2-propanol as hydrogen donor in aqueous buffer (Table 1). Baker's yeast and a red yeast (*Rhodotorula*) preparation showed insufficient activity (entries 1 – 3) as did a variety of bacteria, such as *Lactobacillus*, *Corynebacterium*, *Methylobacterium* and *Streptomyces* sp. (entries 4 – 8), although for a related *Lactobacillus* strain, namely *Lactobacillus kefir*, positive results at 7% v/v 2-propanol were reported in literature.[12f] In contrast, *Mycoplana rubra* (entry 9) showed moderate conversion (44% within 22 h) but good stereoselectivity (93% ee). Best results in terms of activity and stereoselectivity in general were obtained with *Rhodococcus* spp. (entries 10 – 20), except for *Rhodococcus* sp. NCIMB 11216 and *R.* USA-AN-012 both showing low activity. For all active strains the alcohol obtained possessed the (*S*)-configuration.



From the group of *Rhodococci*, *R. ruber* DSM 44541 and *R. ruber* DSM 43338 were selected for the reduction of the positional isomers 3- and 2-acetylpyridine **2a** and **3a**, respectively (Table 2). For both isomeric substrates, the corresponding (*S*)-alcohols (*S*)-**2b** and (*S*)-**3b** were obtained in excellent ee (> 99%) with both strains, while *R. ruber* DSM 43338 showed higher activity. A derivative of **3b** is needed for the synthesis of PNU-142721, a drug candidate for treatment of AIDS.[2a,18] Optically pure 1-(3-pyridyl)-ethanol **2b** was used as starting material for the synthesis of *allo*-heteroyohimbine alkaloids.[19]For both strains, an increase in reaction rate was observed from the *o*- (**3a**) *via* the *m*- (**2a**) to the

Table 1. Screening of micro-organisms for the reduction of 4-acetylpyridine **1a** in the presence of 2-propanol (10% v/v, 22 hours).

Entry	Micro-organism	C [%]	ee_P [%]
1	Baker's yeast (Aströ Co.)	n. c.	–
2	Baker's yeast (Hofer Co.)	3.2	–
3	*Rhodotorula mucilaginosa* DSM 70404	3.4	–
4	*Lactobacillus sakei* DSM 20017	n. c.	–
5	*Corynebacterium glutamicum* ATCC 13032	n. c.	–
6	*Methylobacterium* FCC 031	0.2	–
7	*Mycobacterium paraffinicum* NCIMB 10420	0.1	–
8	*Streptomyces lavendulae* ATCC 55209	n. c.	–
9	*Mycoplana rubra* R14, FCC 009	43.9	93 (*S*)
10	*Rhodococcus* USA-AN-012, FCC 021	2.8	80 (*S*)
11	*Rhodococcus* ANT-AN-037, FCC 011	98.5	> 99 (*S*)
12	*Rhodococcus* SRB-AN-019, FCC 022	39.5	> 99 (*S*)
13	*Rhodococcus* SRB-AN-030, FCC 024	99.7	> 99 (*S*)
14	*Rhodococcus ruber* DSM 43338	99.5	> 99 (*S*)
15	*Rhodococcus erythropolis* DSM 312	99.4	> 99 (*S*)
16	*Rhodococcus* sp. NCIMB 11216	7.5	95 (*S*)
17	*Rhodococcus equi* IFO 3730	99.5	96 (*S*)
18	*Rhodococcus ruber* DSM 44540	99.5	> 99 (*S*)
19	*Rhodococcus ruber* DSM 44541	99.5	> 99 (*S*)
20	*Rhodococcus erythropolis* NCIMB 11539	99.4	> 99 (*S*)

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Table 2. Microbial reduction of 3- and 2-acetylpyridine **2a** and **3a** in the presence of 2-propanol (10% v/v, 42 hours).

Substrate	Micro-organism	Product	C [%]	ee_P [%]
2a	*R. ruber* DSM 43338	(*S*)-**2b**	84	>99
2a	*R. ruber* DSM 44541	(*S*)-**2b**	77	>99
3a	*R. ruber* DSM 43338	(*S*)-**3b**	98	>99
3a	*R. ruber* DSM 44541	(*S*)-**3b**	57	>99

Table 3. Microbial reduction of 3-acetylthiophene **5a** and 2-acetylfuran **7a** in the presence of 2-propanol (10% v/v, 42 hours).

Entry	Micro-organism	3-Acetylthiophene **5a**		2-Acetylfuran **7a**	
		C [%]	ee_P [%]	C [%]	ee_P [%]
1	*Mycoplana rubra* R14, FCC 009	29.5	35 (*S*)	17.9	12 (*S*)
2	*Rhodococcus* ANT-AN-037, FCC 011	4.3	n. d.	n. c.	n. d.
3	*Rhodococcus* SRB-AN-030, FCC 024	21.6	94 (*S*)	15.6	97 (*S*)
4	*Rhodococcus* SRB-AN-019, FCC 022	3.3	n. d.	1.6	n. d.
5	*Rhodococcus equi* IFO 3730	49.6	94 (*S*)	54.5	92 (*S*)
6	*Rhodococcus ruber* DSM 44540	35.6	99 (*S*)	58.5	99 (*S*)
7	*Rhodococcus rube*r DSM 44539	2.4	n. d.	12.2	>99 (*S*)
8	*Rhodococcus* sp. NCIMB 11216	0.4	n. d.	n. d.	n. d.
9	*Rhodococcus erythropolis* NCIMB 11539	51.9	99 (*S*)	26.1	93 (*S*)
10	*Rhodococcus erythropolis* DSM 312	47.9	99 (*S*)	19,7	97 (*S*)
11	*Rhodococcus ruber* DSM 43338	58.5	99 (*S*)	56.8	99 (*S*)
12	*Rhodococcus ruber* DSM 44541	67.1	99 (*S*)	58.7	>99 (*S*)

n. d. not determined, due to too low conversion.

p-isomer (**1a**). It is presumed that this is due to steric hindrance exerted by the proximity of the basic nitrogen, which is heavily hydrated in the aqueous medium.

Surprisingly, none of the 20 organisms listed above were able to reduce the *N*-methylpyrrole derivative **4a**. However, better results were obtained with thiophene analogues. Thus, 3-acetylthiophene **5a** was reduced to the corresponding (*S*)-alcohol by various *Rhodococcus* spp. and *Mycoplana rubra* R14 (Table 3), while the (red) yeasts showed exceedingly low activity (data not shown). As for the pyridine derivatives, *Rhodococcus* sp. NCIMB 11216 showed low activity for **5a**. Again, best results in terms of activity and stereoselectivity were obtained employing *R. ruber* DSM 43338 (entry 11) and *R. ruber* DSM 44541 (99% ee, entry 12), whereby the latter showed better activities. The reduction of 2-acetylthiophene (**6a**) to furnish (*S*)-**6b** proceeded in a comparable manner as for the 3-acetyl derivative **5a** with *R. ruber* DSM 43338 (98% ee, 40% conversion within 42 h).

It is noteworthy that the presence of the heterocyclic sulphur atom did not significantly interfere with the catalytic activity nor the stereoselectivity.

1-(2-Furyl)ethanol **7b** and its derivatives have been utilised as chiral building blocks for the synthesis of numerous natural products, such as carbohydrates, pheromones, alkaloids,[20] and macrolides like macrospelides H, G[21] and A, B.[22] A screening to obtain this building block from the corresponding ketone **7a** by biocatalytic hydrogen transfer revealed, that *R. ruber* DSM 44540, DSM 44541 and *R. ruber* DSM 43338 (Table 3, entries 6, 12, 11) gave the highest conversion and best enantiomeric purity of alcohol (*S*)-**7b**. Interestingly, some strains showed higher selectivity and activity for 3-acetylthiophene **5a** as compared to 2-acetylfuran **7a** (entries 9–11), and *vice versa* (entry 6, activity; entry 3: selectivity), no clear trend was observable.

In the small-scale biotransformations described above, the substrate concentration was always 9 g L^{-1}, which corresponds to approx. 74 to 81 mmol L^{-1} depending on the substrate. Since for preparative-scale transformations higher substrate concentrations are desired, the conversion of pyridyl derivatives **1a**–**3a** using *R. ruber* DSM 44541 and *R. ruber* DSM 43338 was investigated at substrate concentrations between 100 and 700 mmol L^{-1} after 17 h of reaction time (Figure 1). As observed during the screening, 4-acetylpyridine **1a** was transformed fastest, while 2-acetylpyridine **3a** was slowest. Both organisms tolerated a substrate concentration of up to 700 mmol L^{-1}, which corresponds to 84 g L^{-1}. Depending on the substrate, the highest productivity was within a range of 150–400 mmol L^{-1}.

In order to prove the applicability of the method on a preparative scale, the bioreduction of 3-acetyl-pyridine **2a** was performed with 623 mg **2a** (270 mmol L^{-1}, 32 g L^{-1}) in the presence of 10% v/v 2-propanol employing *R. ruber* DSM 44541. After 48 hours, 412 mg of (*S*)-1-

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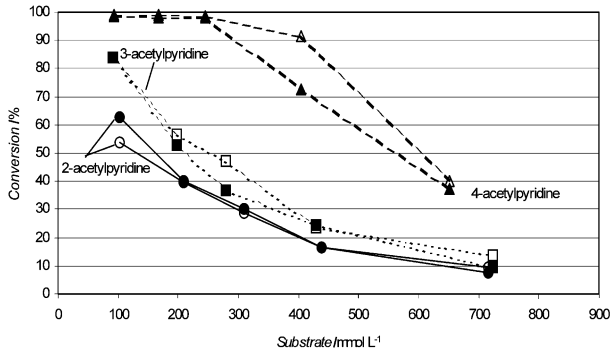

Figure 1. Conversion from the reduction of 2-, 3- and 4-acetylpyridyl isomers with increasing substrate concentration after 17 h. Open symbols: *R. ruber* DSM 44541, solid symbols: *R. ruber* DSM 43338; –Δ–: 4-acetylpyridine **1a**, –□–: 3-acetylpyridine **2a**, –○–: 2-acetylpyridine **3a**.

(4-pyridyl)-ethanol **2b** (75% conversion, 65% isolated yield, >99% ee) were obtained.

Conclusions

Biocatalytic reduction of heteroaryl methyl ketones employing *Rhodococcus* spp. *via* hydrogen-transfer at the expense of 2-propanol as hydrogen donor proved to be a highly efficient method for the preparation of the corresponding heteroaryl methylcarbinols in high chemical and optical yields. The predictability of the stereochemical outcome according to Prelog's rule was excellent and always furnished the corresponding (*S*)-alcohols.

Whereas *Mycoplana rubra* showed moderate activity and stereoselectivity, a range of *Rhodococcus* spp. were identified, which reduced heteroaryl methyl ketones with excellent stereoselectivity (>98% ee). Among them, *Rhodococcus ruber* DSM 44541 proved to be the champion with respect to activity and selectivity. Strains of the genus *Rhodococcus erythropolis* are also selective (>93% ee), however, they were less active on 2-acetylpyridine and 2-acetylfuran. The stereo-electronic properties of the heteroaromatic system had some effect on the reaction rate, but little influence on the stereoselectivity. Good reaction rates can be maintained at substrate concentrations of up to 400 mmol L^{-1} in an environmentally friendly aqueous-organic solvent mixture at room temperature and thus the reaction provides a practical and green tool for the synthesis of enantiopure 1-heteroarylethanols.

Experimental Section

Ketones **1a**–**7a** are commercially available. For analytical purposes, samples of the corresponding *rac*-alcohols **1b**–**7b** were synthesised by reduction of the ketones ($NaBH_4$, MeOH, 5 °C), spectroscopic data were consistent with those reported in the literature (**1b**–**3b**,[10f] **4b**,[23] **5b**,[24] **6b**,[25] **7b**[26]).

Baker's yeast was employed as obtained from the Hofer and Aströ Co. (Graz), lyophilised cells of all other micro-organisms were prepared as previously described.[27] *Rhodocccus* spp. *Rhodococcus* ANT-AN-037, FCC 011, *Rhodococcus* SRB-AN-030, FCC 024 and *Rhodococcus* SRB-AN-019, FCC 022 were kindly donated by A. T. Bull (Canterbury, UK) and *Mycoplana rubra* R14, FCC 009 was a gift of G. Braunegg (Graz University of Technology). FCC numbers refer to our in-house Fab-Crew-Culture-Collection. All other strains were obtained from culture collections.

General Procedure for the Screening

The heteroaryl methyl ketone (**1a**–**7a**, 5.0 mg) dissolved in 2-propanol (55 μL) was mixed with a suspension of rehydrated (30 min) lyophilised cells (40 mg) in phosphate buffer (0.50 mL, pH 7.5, 50 mM) in 1.5 mL Eppendorf vials. The mixtures were shaken horizontally for 42 h (30 °C, 130 rpm) and afterwards extracted with ethyl acetate (0.7 mL). The organic phase was dried (Na_2SO_4) and analysed by GC (see Supporting Information).

Conversion at Elevated Substrate Concentration

The screening-procedure was performed as described above with varying amounts of *o*-, *m*- and *p*-acetylpyridine **1a**–**3a** as indicated in Figure 1.

Preparative Biocatalytic Reduction

Lyophilised cells (2.30 g) of *R. ruber* DSM 44541 were rehydrated in phosphate buffer (15 mL, 50 mM, pH 7.5) for 30 minutes (30 °C, 130 rpm). 3-Acetylpyridine **2a** (623 mg, 5.14 mmol) dissolved in 2-propanol (2 mL) was added and the mixture was shaken at 30 °C and 130 rpm for 48 hours. Ethyl acetate (15 mL) was added, the mixture was centrifuged, the phases were separated and the aqueous layer was again extracted with ethyl acetate (2 × 15 mL). The combined organic layers were dried (Na_2SO_4), the solvent evaporated under reduced pressure and the crude product was purified by column chromatography on silica gel to give (*S*)-1-(4-pyridyl)-ethanol (412 mg, yield: 65%, ee > 99%).

The absolute configurations of the heteroaryl methylcarbinols **1b**–**3b** and **5b**–**7b** were determined by comparison of optical rotation values with literature data (see Supporting Information).

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