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# Asymmetric reduction of aryl imines using *Candida parapsilosis* ATCC 7330

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**Abstract**—A highly enantioselective one pot, novel biocatalytic method for the asymmetric reduction of aryl imines is reported. Treatment of aryl imines with *Candida parapsilosis* ATCC 7330 in aqueous medium produces the enantiomerically pure (*R*)-secondary amines in moderate to good yields (55–80%) with excellent enantiomeric excesses (95–>99%).


## 1. Introduction

Asymmetric reduction of C=N bond to the corresponding enantiomerically pure amines is a very useful transformation because enantiomerically pure amines are important precursors to compounds that are of much interest in the pharmaceutical and agricultural industries.[1] For example, enantiomerically pure *N*-(1-phenylethyl)benzenamine is an important chiral intermediate, which finds widespread use in the synthesis of anticholinergic drugs such as desoxyeseroline, physostigmine and esermethole.[2,3] While the reduction of the C=N bond is well established,[4] reports on the asymmetric reduction of C=N are comparatively rare. These enantioselective reductions generally employ catalysts derived from transition metals in low-oxidation states.[5–9] Enantioselective reduction of imines using polymethylhydrosiloxane (PMHS) as a hydrogen source in the presence of a chiral ligand with binaphthol and metal catalysts such as $Sn(OTf)_2$, $Zn(OTf)_2$, $In(OTf)_3$, $Cd(CHB)_2$ is known to give reasonable yields (50–99%) but poor enantiomeric excesses of the product amine (29–60% ee).[10] Asymmetric reduction of ketimines with trichlorosilane activated with *N*-methyl L-valine derived Lewis base organocatalyst (up to 92% ee) and *N*-formylproline derivatives (up to 66% ee) has been reported.[11,12] The enantioselective reduction of imines catalyzed by Bronsted acid using a Hantzsch dihydropyridine as the hydrogen source gives yields of 46–91% and ee of 68–98%.[13] The biocatalytic asymmetric reduction of C=O bond is a very well-known reaction,[14] but the asymmetric reduction of imines by enzymes is very rare in the literature in comparison to the chemical methods that have been reported. The enzyme, NAD(P)-dependent oxidoreductase isolated from *Pseudomonas putida* ATCC 12633, catalyzes the reduction of cyclic imines such as 1-piperideine-2-carboxylate and 1-pyrroline-2-carboxylate to L-pipecolate and L-proline, respectively, but the yields and ee are not reported.[15] Imine reductase activity was found in the anaerobic bacterium *Acetobacterium woodii* by screening a dynamic combinatorial library of virtual imine substrates, using a biphasic water–tetradecane solvent system.[16] Clearly, these studies were not directed towards developing biocatalytic methods for the asymmetric reduction of imines. As part of our constant effort in 'green' chemistry towards developing efficient biocatalytic methods for organic transformations, we attempted the asymmetric reduction of imines using *Candida parapsilosis* ATCC 7330. This biocatalyst is well known for the asymmetric reduction of α- and β-keto esters and deracemization of α- and β-hydroxy esters as reported by us.[17–23] The obvious chemical analogy between the reduction of a carbonyl group (C=O) and an imine (C=N) also extends the scope of the biocatalyst, *C. parapsilosis* ATCC 7330. Herein we report a benchmark for the biocatalytic asymmetric reduction of imines.

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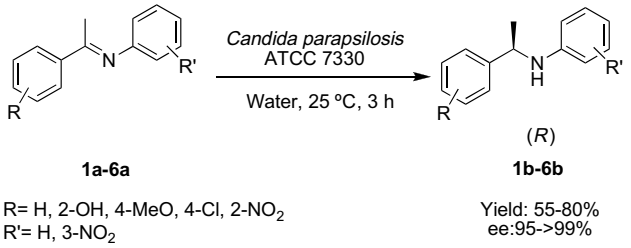
## 2. Results and discussion

Biocatalytic asymmetric reduction of aromatic imines **1a**–**6a** (Scheme 1, Tables 1 and 2) by whole cells of *C. parapsilosis* ATCC 7330 gave the corresponding secondary aromatic amines **1b**–**6b** (Scheme 1, Tables 1 and 2) in 95–>99% ee and 55–80% yields. As can be seen in Table 1, the reduction of unsubstituted (*E*)-*N*-(1-phenylethylidene)benzenamine **1a** (Scheme 1, Tables 1 and 2) using *C. parapsilosis* ATCC 7330 showed high ee (98%) and good yield (71%) of the reduced product. Compounds **5b** and **6b** (Scheme 1, Tables 1 and 2) with the electron-withdrawing substituents (2-$NO_2$ and 3-$NO_2$) were formed in high ee (>99% and 98%) in moderate yields (59% and 55%). Compound **2b** with the 2-hydroxy substituent (Scheme 1, Tables 1 and 2) was formed in high ee (99%) and marginally better yield (65%). Compounds **3b** and **4b** with electron-releasing substituents (4-MeO and 4-Cl) (Scheme 1, Tables 1 and 2) were formed in high ee (97% and 95%) and excellent yields (80% and 74%), respectively. The nature (electron-withdrawing or electron-donating) and position of the substituent on the aromatic ring with 4-chloro, 4-methoxy, 2-hydroxy, 2- and 3-nitro groups does not seem to influence the enantiomeric excesses of the reduced products. This is the first report of the preparation of enantiomerically pure chiral amines **1b**–**6b** (Scheme 1, Tables 1 and 2) by the asymmetric reduction of prochiral imines using whole cells. Thus, whole cells of *C. parapsilosis* ATCC 7330 catalyzes the formation of enantiomerically pure secondary aromatic amines, that is, **1b**–**6b** with excellent enantiomeric excesses (95–>99%) and high yields (55–80%). Since the reaction is carried out in an aqueous medium and no cofactor is added, it is an attractive process for the preparation of enantiomerically pure secondary aromatic amines. The absolute configurations of amines **1b**, **3b** and **4b** were assigned to be (*R*) by comparing their specific rotation values with those reported in the literature.[11] Interestingly, as reported by us earlier,[18,21] this biocatalyst *C. parapsilosis* ATCC 7330 reduces the prochiral keto esters [α and β] to the (*S*)-enantiomer and it also has an (*R*) specific oxidoreductase, which is involved in the deracemization.[20,21] The specific rotation values of amines **2b**, **5b** and **6b** are reported here for the first time. Compounds **2b**, **5b** and **6b** had the same elution profile as compounds **1b**, **3b** and **4b**; the (*R*)-enantiomer is the early eluting enantiomer and the (*S*)-enantiomer is the late eluting enantiomer (Table 2).




**Scheme 1.** Asymmetric reduction of aromatic imines **1a**–**6a** using the whole cells of *Candida parapsilosis* ATCC 7330.

## 3. Conclusions

Enantiomerically pure (*R*) secondary amines were obtained with excellent ee (up to >99% ee) and good yields (up to 80%) using *C. parapsilosis* ATCC 7330 as a biocatalyst. Substrates with electron-withdrawing and electron-donating groups also gave good ee and yields. Mild reaction conditions (pH 6.8, 25 °C) and no additional requirement of the addition of cofactors make this water-based biotransformation mediated by *C. parapsilosis* ATCC 7330 a very

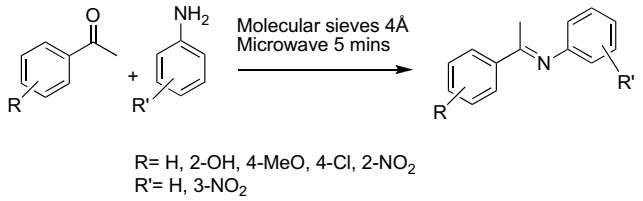
**Table 1.** Asymmetric reduction of aryl imines **1a**–**6a** using whole cells of *Candida parapsilosis* ATCC 7330

| Product number | R | R′ | ee[a] (%) | Yield (%) | $[\alpha]_D^{25}$ $CHCl_3$ | Abs. Config. |
|---|---|---|---|---|---|---|
| **1b** | H | H | 98 | 71 | −11.4 (*c* 1.1) | (*R*)[b] |
| **2b** | 2-OH | H | 99 | 65 | −8.1 (*c* 1.3) | (*R*)[c] |
| **3b** | 4-MeO | H | 97 | 80 | −3.97 (*c* 0.8) | (*R*)[b] |
| **4b** | 4-Cl | H | 95 | 74 | −11.9 (*c* 1.2) | (*R*)[b] |
| **5b** | 2-$NO_2$ | H | >99 | 59 | − 7.9 (*c* 1.0) | (*R*)[c] |
| **6b** | H | 3-$NO_2$ | 98 | 55 | − 5.4 (*c* 0.6) | (*R*)[c] |

[a] Enantiomeric excess was determined by Chiral HPLC.
[b] The absolute configurations of **1b**, **3b** and **4b** were found to be (*R*) by comparing the $[\alpha]_D^{25}$ values with the reported literature data.[11]
[c] Compounds **2b**, **5b** and **6b** had the same elution profile as compounds **1b**, **3b** and **4b**, that is, the (*S*)-enantiomer is the late eluting enantiomer, while the (*R*)-enantiomer is the early eluting enantiomer (see Table 3).

**Table 2.** Retention times of enantiomerically pure aromatic amines **1b**–**6b**

| Product number | R | R′ | Chiral column | Elution of HPLC peaks (retention time in minutes) | |
|---|---|---|---|---|---|
| | | | | Early (major) | Later (minor) |
| **1b** | H | H | ODH | 14.7 | 17.1 |
| **2b** | 4-Cl | H | ODH | 15.3 | 18.7 |
| **3b** | 4-OMe | H | ODH | 17.5 | 19.7 |
| **4b** | 2-OH | H | OJH | 16.7 | 19.3 |
| **5b** | 4-$NO_2$ | H | OJH | 24.7 | 29.0 |
| **6b** | H | 3-$NO_2$ | ODH | 19.3 | 22.7 |




**Scheme 2.** Synthesis of aryl imines **1a**–**6a**.

**Table 3.** Synthesis of aryl imines **1a**–**6a**

| Product number | R | R′ | Yield (%) |
|---|---|---|---|
| **1a** | H | H | 57 |
| **2a** | 2-OH | H | 70 |
| **3a** | 4-MeO | H | 61 |
| **4a** | 4-Cl | H | 69 |
| **5a** | 2-$NO_2$ | H | 72 |
| **6a** | H | 3-$NO_2$ | 70 |

efficient asymmetric reduction reaction of imines to the corresponding amines. Moreover, its operational simplicity makes this procedure extremely attractive and a practical alternative to the existing methods, and we believe this will find general acceptance in organic synthesis.

## 4. Experimental

### 4.1. General methods

*C. parapsilosis* ATCC 7330 was purchased from ATCC, Manassas, VA 20108, USA. All chemicals used for media preparation were purchased locally. All substrates were synthesized using the reported method[24] modified by us (refer Scheme 2, Table 3 and Experimental Section 4.2). $^1$H and $^{13}$C NMR spectra were recorded in $CDCl_3$ on a JEOL GSX400 and Bruker AV-400 spectrometers operating at 400 MHz and 100 MHz. Chemical shifts are expressed in ppm values using TMS as an internal standard. Infrared spectra were recorded on a Shimadzu IR 470 Instrument. Mass spectra were recorded on a Q TOF micromass spectrometer. The enantiomeric excess (ee) was determined by HPLC analysis using a chiral column on a Jasco PU-1580 liquid chromatograph equipped with PDA detector. The chiral columns used were Chiralcel OD-H and Chiralcel OJ-H (Daicel, 4.6 × 250 mm). The solvent used was hexane/isopropanol (98:2) at a flow rate of 1 ml min$^{-1}$ and the absorbance monitored using a PDA detector at 254 nm. Optical rotations were determined on an Autopal® digital polarimeter. TLC was carried out using Kieselgel 60 F254 aluminium sheets (Merck 1.05554).

### 4.2. Synthesis of aryl imines

Condensation of acetophenone (4.0 mmol, 500 mg) and aniline (6.0 mmol, 0.6 ml) was carried out using a domestic microwave oven (Power 30) for 5 min. Molecular sieves 4 Å were used to remove the side product, water, to facilitate the completion of the reaction. After completion, the reaction mixture was neutralized with dil. HCl (1 M), extracted with ethyl acetate (3 × 10 ml), dried and concentrated. Compound (*E*)-*N*-(1-phenylethylidene)benzenamine **1a** (Scheme 2 and Table 3) was obtained as a very pale yellow solid in 57% yield (0.449 mg, 2.2 mmol) after deactivated silica gel column purification using hexane/ethyl acetate (99:1) as solvent. The same procedure was followed for compounds **2a**–**6a** (Scheme 1 and Table 1).

### 4.3. Asymmetric reduction of various aryl imines 1a–6a

**4.3.1. Growth of *C. parapsilosis* ATCC 7330.** Cells of the yeast *C. parapsilosis* ATCC 7330 were grown as reported earlier[25] and used for the asymmetric reduction.

**4.3.2. A typical experimental procedure for the asymmetric reduction of (*E*)-*N*-(1-phenylethylidene)benzenamine 1a using the whole cells of *C. parapsilosis* ATCC 7330.** To the harvested cells (18 g) suspended in distilled water [16.2 ml], (*E*)-*N*-(1-phenylethylidene)benzenamine **1a** (Scheme 2 and Table 2) (36 mg, 0.182 × 10$^{-3}$ moles) dissolved in ethanol (900 µl) was added. The biotransformation was carried out for 3 h at 150 rpm and 25 °C. Control experiments were carried out wherein only the solvent (ethanol) was added to the cell suspension. After 3 h, the product *N*-(1-phenylethyl)benzenamine **1b** (Scheme 1 and Table 1) was extracted using ethyl acetate (3 × 2 ml). The organic phase was then dried over anhydrous $Na_2SO_4$ and concentrated under vacuum. The other imine compounds **2a**–**6a** (Scheme 1 and Table 1) were also used as substrates in the same manner. The experiments with the imine substrates **1a**–**6a** (Scheme 1 and Table 1) were done in duplicates. The ee of amines **1b**–**6b** (Scheme 1 and Tables 1 and 2) was determined by chiral HPLC using Chiralcel OD-H and OJ-H columns using hexane/isopropanol (98:2) as the mobile phase.

**4.3.3. Spectral data for compounds 2b, 5b and 6b**

**4.3.3.1. (*R*)-*N*-(1-(2-Hydroxyphenyl)ethyl)benzenamine 2b.** Yellow liquid; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ ppm: 1.5 (d, 3H), 4.4 (q, 1H), 6.68–7.13 (m, 9H); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ ppm: 21.0, 45.5, 113.0, 115.1, 116.1, 121.0, 128.0, 128.4, 130.0, 131.3, 147.2, 154.0; IR $\nu_{max}$: 3332.5, 2981.5, 1728.2, 1602.1, 1499.0, 1372.6, 1238.4, 1043.2, 751.4 cm$^{-1}$; HRMS(ESI): found 214.1236, $C_{14}H_{16}NO$ $(M+H)^+$ requires 214.1232.

**4.3.3.2. (*R*)-*N*-(1-(2-Nitrophenyl)ethyl)benzenamine 5b.** Yellow liquid; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ ppm: 1.5 (d, 3H), 4.4 (q, 1H), 6.36–8.15 (m, 9H); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ ppm: 24.9, 53.3, 113.2, 117.9, 123.7, 124.0, 126.1, 126.6, 129.2, 137.8, 147.7, 148.5; IR $\nu_{max}$: 3379.8, 2924.6, 1628.6, 1522.5, 1350.1, 815.5, 794.6, 735.4, 670.7 cm$^{-1}$; HRMS(ESI): found 243.1130, $C_{14}H_{15}N_2O_2$ $(M+H)^+$ requires 243.1134.

**4.3.3.3. (*R*)-3-Nitro-*N*-(1-phenylethyl)benzenamine 6b.** Yellow liquid; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ ppm: 1.4 (d, 3H), 4.4 (q, 1H), 6.66–7.54 (m, 9H); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ ppm: 29.6, 53.4, 109.0, 112.3, 113.1, 117.8, 120.5, 125.7, 128.8, 129.3, 129.8, 147.3; IR $\nu_{max}$: 3375.5, 2921.2, 1736.0, 1628.7, 1523.3, 1350.3, 1259.8, 794.7, 734.7 cm$^{-1}$; HRMS(ESI): found 243.1136, $C_{14}H_{15}N_2O_2$ $(M+H)^+$ requires 243.1134.

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