Asymmetric Bioreduction of a Bulky Ketone: 1-Phenyl-1-(2-phenylthiazol-5-yl)-methanone

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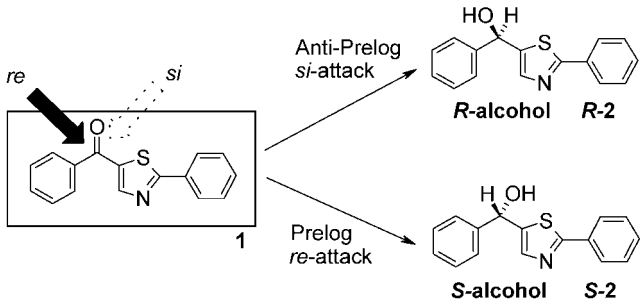
Abstract: We have investigated the preparation of (*R*)- and (*S*)-1-phenyl-1-(2-phenylthiazol-5-yl)-methanol by asymmetric bioreduction of the corresponding bulky ketone 1-phenyl-1-(2-phenylthiazol-5-yl)-methanone with whole microbial cells. A short screening of 38 microbial strains allowed the selection of two suitable yeast strains fulfilling enantiocomplementarity. Gram-scale preparations of the *S*- and *R*-alcohols were achieved with high yield and high optical purity using, respectively, *Saccharomyces montanus* CBS 6772 (yield 79%, ee 96%) and *Rhodotorula glutinis* var. *dairenensis* MUCL 30607 (yield 96%, ee 91%). The two enantiomeric alcohols prepared are novel compounds described here for the first time.

Keywords: biocatalysis; bioreduction; enantioselectivity; fermentation; yeast strains

Introduction

Optically active alcohols are potentially useful chiral building blocks. Thus, the asymmetric reduction of prochiral ketones using biocatalysts has been extensively explored and is now one of the outstanding synthetic methods to prepare enantiopure secondary alcohols.[1-6]

The core objective of this work was first to investigate the asymmetric reduction of the prochiral ketone **1** by whole-cell biocatalysts and second to establish a simple and rapid procedure for the gram-scale preparation of the two corresponding alcohols *S*-**2** and *R*-**2** using different microbial strains (Scheme 1). The substrate is a hindered ketone, the carbonyl group is indeed surrounded by two bulky aromatic substituents, a phenyl group and a 2-phenylthiazol-5-yl moiety. Among the wide range of ketones successfully reduced by biocatalytic methods and leading to chiral secondary alcohols, only few examples of bioreduction of diaromatic ketones have been reported.[7-14]



Scheme 1. Stereoselective reduction of the ketone **1**.

Taking into account that the 2-phenylthiazol-5-yl moiety is bulkier than the phenyl moiety, the reduction of the prochiral ketone **1**, with Prelog specificity[15] implying a "*re*-face attack," will provide the corresponding *S*-alcohol; in contrast, anti-Prelog specificity with an "*si*-face attack" will lead to the corresponding *R*-alcohol (Scheme 1). Previous bioreduction studies have shown that microorganisms with a Prelog specificity are prevalent, while those with an anti-Prelog specificity are to some extent less common.[2]

Results and Discussion

If we first examine the results of the microbial screening, summarized in Table 1, in terms of reduction yields, among the 38 microorganism strains evaluated, 9 of them catalyzed the reduction of the ketone **1** to the corresponding alcohol **2** with a yield higher than 50%. Within the strains showing a high reduction yield (> 50%), only yeast strains achieve high enantioselectivity (ee >80%). Three yeast strains, *Rhodotorula mucilaginosa, Rhodotorula rubra* ATCC 4056, and *Saccharomyces montanus* CBS 6772 reduced the ketone **1** to the *S*-alcohol with enantiomeric excesses of 81 –

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Table 1. Results of the microbial screening.

Entry	Strain	Time (days)	Reduction yield (%)	Enantiomeric excess (%) (Absolute configuration)
1	*Absidia cylindrospora* LCP 57-1569[a]	11	0	-
2	*Acremonium alternatum* MMP 3010[a]	11	<5	nd
3	*Aspergillus alliaceus* NRRL 315[a]	14	0	-
4	*Beauveria bassiana* ATTC 7159[a]	14	<5	nd
5	*Cunninghamella echinulata* NRRL 3655[a]	10	0	-
6	*Cunninghamella echinulata* var. *elegans* ATCC 9245[a]	14	0	-
7	*Curvularia lunata* NRRL 2380[a]	14	<5	nd
8	*Fusarium roseum* ATCC 14717[a]	14	0	-
9	*Geotrichum candidum* LCP 98-4202[a]	14	<5	nd
10	*Mortierella isabellina* LCP 52-108[a]	7	55[d]	2 (*R*)
11	*Mucor plumbeus* CBS 110-16[a]	10	<5	nd
12	*Rhizopus arrhizus* ATCC 11145[a]	7	39[d]	58 (*S*)
13	*Rhizopus stolonifer* ATCC 6227b[a]	7	70[d]	7 (*S*)
14	*Mucor janssenii* NRRL 3628[a]	7	68[d]	7 (*R*)
15	*Pseudomonas fluorescens* CIP 6913[b]	14	0	-
16	*Rhodococcus erythropolis* ATCC 4277[b]	14	0	-
17	*Streptomyces olivaceus* NRRL 1125[b]	14	0	-
18	*Streptomyces aureofaciens* ATCC 10762[b]	14	0	-
19	*Pseudomonas putida* ATCC 29607[b]	14	0	-
20	*Streptomyces griseolus* ATCC 3325[b]	14	<5	98 (*S*)
21	*Streptomyces griseus* NRRL 13150[b]	14	0	-
22	*Streptomyces rimosus* NRRL 2234[b]	14	<5	nd
23	*Streptomyces vinaceus* ATCC 11861[b]	14	0	-
24	*Rhodococcus rhodochrous* DSM 43198[b]	14	<5	nd
25	*Candida parapsilosis* CBS 6318[c]	7	<5	nd
26	*Candida tropicalis* NRRL Y2001[c]	7	<5	nd
27	*Kloeckera magna* NRRL Y1091[c]	2	<5	nd
28	*Pichia anomala* NRRL Y40[c]	7	17	43 (*S*)
29	*Rhodotorula glutinis* NRRL Y1091[c]	2	77	52 (*R*)
30	*Saccharomyces cerevisiae*[c]	7	<5	nd
31	*Rhodosporidium toruloides* MUCL 30328[c]	2	48	94 (*S*)
32	*Rhodotorula buffonii* MUCL 29812[c]	2	3	60 (S)
33	*Rhodotorula minuta* MUCL 30637[c]	2	3	12 (*S*)
34	*Rhodotorula mucilaginosa* L[c]	2	80	81 (*S*)
35	*Rhodotorula rubra* ATCC 4056[c]	1	76	80 (*S*)
36	*Saccharomyces montanus* CBS 6772[c]	2	92	98 (*S*)
37	*Rhodotorula glutinis* var. *dairenensis* MUCL 30607[c]	2	90	85 (*R*)
38	*Rhodotorula pilimanae* MUCL 27811[c]	2	62	18 (R)

[a] Filamentous fungi cultivation medium composition (g/L): corn steep liquor (10.0), $MgSO_4 \cdot 7\,H_2O$ (0.5), $NaNO_3$ (2.0), $FeSO_4 \cdot 7\,H_2O$ (0.02), KCl (0.5), glucose (3.0), K_2HPO_4 (0.2), and K_2HPO_4 (0.1).

[b] Bacteria cultivation medium composition (g/L): yeast extract (5.0), soy bean peptone (5.0), NaCl (5.0), K_2HPO_4 (5.0), and glucose (20.0), adjusted to pH 7 with 1 N HCl.

[c] Yeasts cultivation medium composition (g/L): yeast extract (5.0), soy bean peptone (5.0), malt extract (10.0), and glucose (20.0).

[d] In these cases, the substrate is solubilized in 3 mL of a 20% Tween 80®/acetone solution.
nd: yield was too low to determine ee.

98%. One yeast strain, *Rhodotorula glutinis* var. *dairenensis* MUCL 30607 exhibited an enantiocomplementarity behavior producing the *R*-alcohol with an enantiomeric excess of 85%. Baker's yeast (*Saccharomyces cerevisiae*), commonly reported as the prime reduction biocatalyst, is here inefficient in the reduction of the ketone **1**, the steric bulkiness around the carbonyl might be the reason for this lack of reactivity, as mentioned in other cases.[16]

In terms of stereoselectivity, if we consider enantiomeric excesses higher than 80%, five strains [*Rhodotorula mucilaginosa* (81%), *Rhodotorula rubra* ATCC 4056 (80%), *Rhodosporidium toruloides* MUCL 30328 (94%), *Saccharomyces montanus* CBS 6772 (98%), *Streptomyces griseolus* ATCC 3325 (98%)] follow "Prelog's rule" leading to the *S*-alcohol and only one strain [*Rhodotorula glutinis* var. *dairenensis* MUCL 30607 (85%)] shows an anti-Prelog specificity leading to the *R*-alcohol. These results are coherent with "Prelog's rule", this simple model states that the majority of dehydrogenases deliver the hydride ion to the *re*-face of a pro-chiral ketone.[2]

The best strains selected for further preparative synthesis of the *S*- and the *R*-alcohols are respectively *Saccharomyces montanus* CBS 6772 (yield 92%, ee 98%), and *Rhodotorula glutinis* var. *dairenensis* MUCL 30607 (yield 90%, ee 85%). In order to improve the bioreduction yield and the stereoselectivity of these two yeasts, we have investigated the effect of the substrate **1** concentration on the bioreduction performance. The results of this study appear in Tables 2 and 3 and in Figure 1.

Table 2. Reduction by *Saccharomyces montanus* CBS 6772: effect of the ketone **1** concentration and of glucose addition on the synthesis of the *S* alcohol, *S*-**2**.

Entry	[Ketone] (g/L)	Addition of a glucose solution (20g/L)	Time (days)	Reduction yield (%)	Enantiomeric excess (%)	Final concentration of *S*-Alcohol (g/L)
1	0.3	-	2	92	98	0.28
2	0.5	-	2	90	97	0.45
3	0.8	-	3	63	97	0.51
		+5 mL with substrate addition	3	79	97	0.64
4	1.0	-	3	63	97	0.63
		+5 mL with substrate addition +5 mL after 3 days of biotransformation	4	77	97	0.77
5	1.5	-	7	64	96	0.97
		+5 mL with substrate addition +5 mL after 3 days of biotransformation	7	79	96	1.20
6	2.0	-	7	49	96	0.99
		+5 mL with substrate addition +5 mL after 3 days of biotransformation	7	52	96	1.05
7	3.0	+5 mL with substrate addition +5 mL after 3 days of biotransformation	7	31	96	0.94
8	4.0	+10 mL with substrate addition +10 mL after 3 days of biotransformation	7	30	96	1.21
9	5.0	+10 mL with substrate addition +10 mL after 3 days of biotransformation	7	23	96	1.16

The stereoselectivity of the oxido-reductase system of *Saccharomyces montanus* CBS 6772, leading to the *S* enantiomer, is mostly unaffected by the variation of the substrate concentration (0.3 to 5.0 g/L). The enantiomeric excesses obtained are all ranged between 98% and 96% (Table 2.).

In contrast, the stereoselectivity of *Rhodotorula glutinis* var. *dairenensis* MUCL 30607, leading to the *R* enantiomer, is markedly dependent on the substrate concentration. The enantiomeric excess decreases dramatically from 91% to 29% for substrate concentration values from 0.1 to 0.5 g/L (Table 3.). This might be due to the presence in *Rhodotorula glutinis* var. *dairenensis* MUCL 30607 of different dehydrogenases,[17] possessing opposite stereochemical specificities and different affinities, which compete for the reduction of the substrate **1**. As a consequence, a lower concentration (0.1 g/L) of ketone **1** was chosen for the preparation of the *R*-alcohol (yield 96%, ee 91%) without any supplementary addition of glucose. It would be interesting, for further synthesis of this *R*-alcohol, in order to obtain a higher final concentration of *R*-2 to use an optimized semi-batch procedure maintaining the substrate **1** concentration below the critical value of 0.1 g/L.

The results summarized in Figure 1 and Table 2 show that the final concentration of the produced *S*-alcohol *S*-**2** was three-fold increased for ketone **1** con-

Table 3. Reduction by *Rhodotorula glutinis* var. *dairenensis* MUCL 30607: effect of the ketone **1** concentration on the synthesis of the *R* alcohol, *R*-**2**.

Entry	[Ketone] (g/L)	Time (days)	Yield of the reduction (%)	Enantiomeric excess (%)	Final concentration of *R*-Alcohol (g/L)
1	0.1	2	96	91	0.10
2	0.3	2	90	85	0.27
3	0.5	4	80	29	0.40

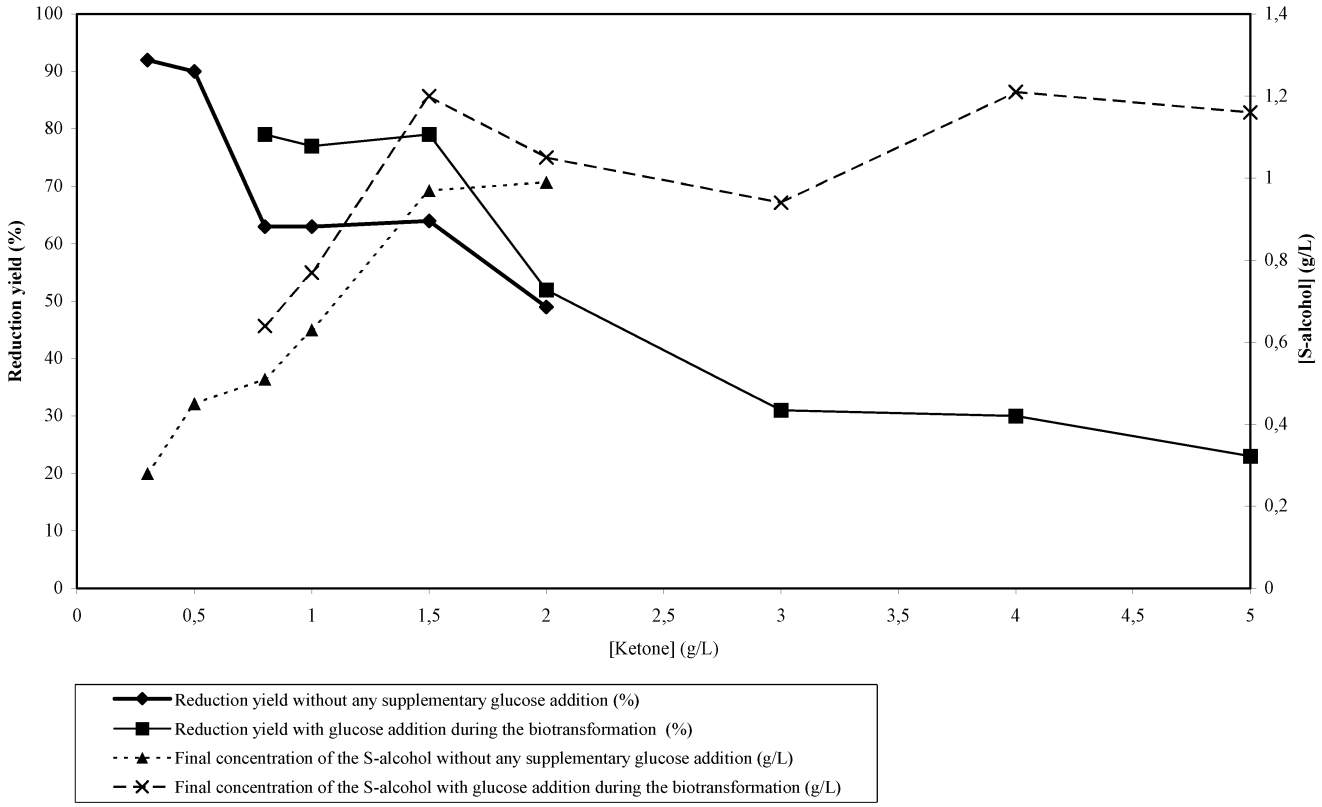

Figure 1. Reduction by *Saccharomyces montanus* CBS 6772: effect of the ketone **1** concentration and of glucose addition on the synthesis of the *S* alcohol, *S*-**2**.

centrations of 0.3 g/L and 1.5 g/L with respective values of 0.28 g/L (entry 1) and 0.97 g/L (entry 5). For ketone **1** concentrations higher than 1.5 g/L, the final concentration of *S*-**2** remained unchanged, there was indeed no significant difference for ketone **1** concentrations of 1.5 g/L (0.97 g/L, entry 5) and 2.0 g/L (0.99 g/L, entry 6) leading to an average final concentration of the *S*-alcohol of 0.98 g/L.

Considering the influence of the addition of glucose (Table 2, entries 3, 4, 5, and 6), for ketone **1** concentrations of 0.8 g/L, 1.0 g/L, and 1.5 g/L, it is clear that the addition of glucose enhances the final concentration of *S*-**2** by about 25%. On the contrary, for ketone **1** concentrations higher than 1.5 g/L, a supplementary addition of glucose had no effect. And the final concentration of *S*-**2** remained stable with an average value of 1.1 g/L (entries 5, 6, 7, and 8). Analysis of these data suggested that the maximum capacity of reduction, under these studied conditions, was limited to a ketone **1** concentration of 1.5 g/L. The best result chosen for the gram-scale preparation of the *S*-alcohol was obtained when employing 1.5 g/L as the initial concentration of ketone **1** with the addition of glucose as a reducing agent (entry 5: yield 79%, ee 96%, [S-**2**] 1.2 g/L).

Conclusion

In summary, the asymmetric bioreduction of the bulky diaromatic ketone **1** has been successfully carried out with fermenting yeasts. This simple and environmentally-friendly method allowed the gram-scale preparation of the *S*- and the *R*-alcohols **2** with high yields and optical purities, using respectively *Saccharomyces montanus* CBS 6772 (yield 79%, ee 96%) and *Rhodotorula glutinis* var. *dairenensis* MUCL 30607 (yield 96%, ee 91%). These results demonstrate that enantiocomplementarity can be achieved even with a very hindered substrate. The two enantiomeric alcohols prepared are novel compounds described here for the first time. These examples are one more proof that the biocatalytic approach is a valuable method for the synthesis of optically active alcohols.

Experimental Section

General Experimental Details

Melting points were measured on a micro-melting point Electrothermal 9300 apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Advance 250 NMR spectro-

meter, chemical shifts are reported in parts per million (ppm, δ) with CD_2Cl_2 as solvent and TMS as an internal standard. Optical rotations were measured on a Perkin-Elmer Polarimeter 341. Enantiomeric excesses were determined by HPLC analysis using a chiral column (Chiralcel OD-H 4.6 × 125 mm, 5 μm) with eluent *n*-heptane-ethanol, 70:30, flow rate of 0.5 mL/min, detection performed at 310 nm. The absolute configuration was determined by X-ray crystallography analysis of the *O*-β-D-glucopyranosyl derivative of the (+)-alcohol obtained by incubation with another fungal strain (Roy and Le Texier, results to be published). Crystallographic data have been deposited with Cambridge Crystallographic Data Centre under the number CCDC 163320. IR spectra were recorded on a Nicolet-Impact 410 spectrophotometer. Mass spectra were obtained on either a VG Platform-Fisons or a Micromass QuattroLC spectrometer.

Synthesis of 1-Phenyl-1-(2-phenylthiazol-5-yl)-methanone (1)

N-Thiobenzoyl-*N*,*N*-dimethylformamide (40.0 g, 208 mmoles, 1.0 equiv.) was dissolved in 320 mL of dichloromethane, followed by the addition of α-bromoacetophenone (41.4 g, 208 mmoles, 1.0 equiv.). This mixture was stirred for 23 hours at room temperature. After cooling at 5 °C, triethylamine (72 mL, 520 mmoles, 2.5 equiv) was slowly added. The resulting mixture was stirred for 2 additional hours and filtered. Methanol was added to the filtrate and this solution was concentrated under reduced pressure until precipitation occurred. After filtration, the collected precipitate was washed several times with methanol affording, after drying, 1-phenyl-1-(2-phenylthiazol-5-yl)-methanone (**1**) as a white powder; yield: 35.2 g (64%). CAS number: [52421-61-1]; mp 124 °C; MS (APcI+): *m/z* = 266 $(M + H)^+$; ^{1}H NMR (CD_2Cl_2, 250 MHz): δ = 8.28 (s, 1H), 8.06 (m, 2H), 7.91 (m, 2H), 7.57 (m, 6H); ^{13}C NMR (CD_2Cl_2, 62.9 MHz): δ = 187.53 (C=O), 174.57, 150.2, 139.3, 138.1, 133.3, 133.2, 131.8, 129.6 (2C), 129.4 (2C), 129.1 (2C), 127.4 (2C); IR (KBr): ν = 1627 cm^{-1} (C=O).

Typical Procedure for the Microbial Screening

Strains were maintained on nutrient agar slants at 4 °C. Erlenmeyer flasks (500 mL for *Streptomyces* sp. and 250 mL for other strains) containing 100 mL of the appropriate sterilized cultivation medium (see Table 1) were inoculated with a glycerol suspension of the tested microorganism and incubated in an orbital shaker (270 rpm) at 27 °C. After 48 h of growth (yeast, bacteria) or 72 h (fungi), the substrate (final concentration 0.3 g/L) was added to this broth in 3 mL of a 10% (or 20%, see Table 1) Tween 80®/acetone solution. The biotransformation was conducted at 270 rpm in an orbital shaker at 27 °C. The biotransformation was monitored by HPLC with a daily sample collection (Macherey-Nagel column, Nucleosil C-18, 250 × 4.6 mm, 5 μm, elution gradient of H_2O:CH_3CN:TFA [60:40:0.1 held for 10 min, 60:40:0.1 to 0:100:0.1 over 20 min, held for 10 min, then returned to 60:40:0.1 over 5 min] at a flow rate of 1 mL/min, detection was performed at 280 and 310 nm; under these conditions the ketone **1** and the alcohol **2** eluted after 22.0 min and 29.5 min, respectively). An aliquot of the broth was centrifuged (4000 rpm, 10 min). The supernatant and a methanolic extract of the cells were daily analyzed. When the bioreduction was completed (longer observation time fixed at 14 days), the incubation mixture was centrifuged (4000 rpm, 10 min). The supernatant was extracted twice with 100 mL of dichloromethane and the cells with 200 mL of acetone. After centrifugation (4000 rpm, 10 min) of the cell extract, the organic layers were pooled and dried over anhydrous magnesium sulfate. This solution was filtered and taken to dryness under vacuum. The residue was dissolved in 100 mL of methanol and this solution was analyzed by HPLC to estimate the final concentration of the alcohol **2**. Finally, alcohol **2** was purified by TLC on silica gel (cyclohexane:ethyl acetate = 7:3 as eluent). The enantiomeric excess of the alcohol **2** was then determined by chiral HPLC analysis.

Synthesis of *S*-1-Phenyl-1-(2-phenylthiazol-5-yl)-methanol (*S*-2)

The bioreduction by *Saccharomyces montanus* CBS 6772 was scaled-up to the gram scale, employing the previously determined operating conditions. 3 Erlenmeyer flasks (2 L) each containing 800 mL of sterilized cultivation medium (see Table 1) were inoculated with a glycerol suspension of *Saccharomyces montanus* CBS 6772 and incubated in an orbital shaker (270 rpm) at 27 °C. After 48 h of cell growth, 40 mL of a glucose solution (20 g/L) and the substrate **1** (1.2 g, 4.53 mmol, final concentration 1.5 g/L) in 35 mL of a 10% Tween 80®/acetone solution were added to each flask. The biotransformation was conducted at 270 rpm in an orbital shaker at 27 °C. After 72 h of biotransformation, 40 mL of a glucose solution (20 g/L) were once more added. The bioreduction was stopped after 7 days. The extraction procedure previously described for the microbial screening was used. The residue was subjected to column chromatography on silica gel (cyclohexane:ethyl acetate = 7:3 as eluent) to afford a white powder of *S*-1-phenyl-1-(2-phenylthiazol-5-yl)-methanol (S-**2**); yield: 2.87 g (10.7 mmol; 79%); mp 115 °C; $[\alpha]_D^{20}$: + 74 (*c* 1.5, CH_2Cl_2) for 98% *ee* (tr = 11 min, Chiralcel OD-H); MS (APcI+): *m/z* = 268 $(M - H^+)$; ^{1}H NMR (250 MHz, CD_2Cl_2): δ = 7.89 (m, 2H), 7.59 (s, 1H), 7.40 (m, 8H), 6.11 (s, 1H), 2.80 (OH, 1H); ^{13}C NMR (62.5 MHz, CD_2Cl_2): δ = 168.8, 143.4, 143.1, 141.1, 134.0, 130.3, 129.2 (2C), 129.0 (2C), 128.6, 126.6(2C), 126.5 (2C), 70.8; IR (KBr): ν = 3436 cm^{-1}.

Synthesis of *R*-1-Phenyl-1-(2-phenyl-thiazol-5-yl)-methanol (*R*-2)

The gram scale bioreduction of ketone **1** by *Rhodotorula glutinis* var. *dairenensis* MUCL 30607 was performed in a bioreactor (30 L fermentor, B.Braun Biostat C). Erlenmeyer flasks (250 mL) containing 100 mL of sterilized cultivation medium (see Table 1) were inoculated with a glycerol suspension of *Rhodotorula glutinis* var. *dairenensis* MUCL 30607 and incubated in an orbital shaker (270 rpm) at 27 °C. After 24 h of cell growth, 150 mL of the shake flask culture were used to inoculate the bioreactor containing 15 L of sterilized yeast cultivation medium (see Table 1). The bioreactor was operated at 27 °C with agitation at 250 rpm and air flow at 15 L/min. After 48 h of growth, the substrate **1** (1.5 g, 5.66 mmol, final concentration 0.1 g/L) in 40 mL of a 10% Tween 80®/acetone solution was added. The biotransformation was completed after 2 days, the product

recovery and purification were similar to those described above. The compound *R*-1-phenyl-1-(2-phenyl-thiazol-5-yl)-methanol (*R*-**2**) was isolated as a white powder; yield: 1.45 g (5.43 mmol; 96%); mp 114 °C; $[\alpha]_D^{20}$: –67 (*c* 1.5, CH_2Cl_2) for 91% ee (tr = 15 min, Chiralcel OD-H); MS (APcI+): *m/z* = 268 ($M - H^+$); ^{1}H NMR (250 MHz, CD_2Cl_2): δ = 7.89 (m, 2H), 7.59 (s, 1H), 7.40 (m, 8H), 6.11 (s, 1H), 2.82 (OH, 1H); ^{13}C NMR (62.5 MHz, CD_2Cl_2): δ = 167.8, 142.4, 142.1, 140.0, 133.0, 129.4, 128.2 (2C), 128.0 (2C), 127.6, 125.6(2C), 125.5 (2C), 69.8 ; IR (KBr): ν = 3429 cm^{-1}.

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