

Studies of the Deracemization of ($\pm$)-2-Hydroxy-1-tetralone by *Trichosporon cutaneum*

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Abstract: The diastereo- and enantioselective bioreduction of ($\pm$)-2-hydroxy-1-tetralone (**6**) to the corresponding enantiopure (1*S*,2*R*)-*cis*-1,2-dihydroxy-1,2,3,4-tetrahydronaphthalene (**1**) (83 % isolated yield, >99 % *ee*), mediated by resting cells of the yeast *Trichosporon cutaneum* CCT 1903 through dynamic kinetic resolution is reported. Deracemization

of ($\pm$)-**6** was observed in kinetic studies on the biotransformation of the enantiomers (*R*)-**6** and (*S*)-**6**.

Keywords: amino alcohols; biotransformations; hydroxytetralone; isomerases; oxidoreductases; *Trichosporon cutaneum*

Introduction

Enantiomerically pure compounds are potentially useful intermediates for the synthesis of a wide variety of bioactive products in the pharmaceutical and agrochemical industries.^[1] Although asymmetric catalysis has undergone an impressive development in the last twenty years, as of today the most common way to obtain enantiomerically pure compounds in industry is still *via* resolution of racemic mixtures.^[2] To obtain a single enantiomer from such a mixture, one can resolve it either by conventional separation techniques or by using an existing difference in reactivity (kinetic resolution).^[3] However, a major limitation of these techniques is that the maximum product yield is only 50 %. The unwanted enantiomer must be separated, racemized and resubmitted to resolution in order to increase this yield. These disadvantages can be overcome by employing dynamic kinetic resolution (DKR). DKR has recently become not only an alternative to traditional kinetic resolution, but also a new procedure for asymmetric synthesis.^[3] It is one of the most useful and reliable methods to prepare a single chiral compound bearing two or more stereocenters starting from a racemate, with a theoretical yield of 100 %. A number of successful examples for DKR methods have been reviewed recently.^[4] Some of them involve solely conventional chemical methods,^[5]

whereas others combine chemical and biocatalytic steps.^[6] In the latter case, enzymes or whole cells contribute either to racemization^[7] or to kinetic resolution.^[4]

Following our research on DKR,^[8] we now report the diastereo- and enantioselective preparation of (1*S*,2*R*)-1,2-dihydroxy-1,2,3,4-tetrahydronaphthalene (**1**). The chiral *cis*-(1*S*,2*R*)-diol **1** can be a valuable intermediate as a building block in asymmetric organic synthesis. Furthermore, diols **1** and **2** are excellent precursors to the *cis*-amino alcohol **3**, which can be easily obtained through a Ritter reaction or by the methodology of Resnick et al. (Figure 1).^[9] The amino alcohol **3** has been used as a chiral ligand in the cata-

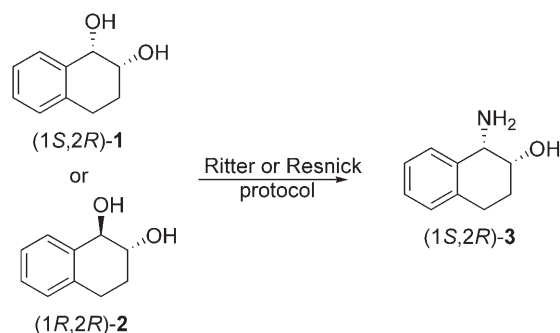


Figure 1.

lytic reduction of ketones and as a toolbox to probe ligand conformations in transition metal-catalyzed reactions (Figure 2, compound **5**).^[10,11] The *cis*-(1*R*,2*S*)-diol **1** has been utilized as chiral intermediate for the synthesis of the chiral crown ether (*S*,*R*,*R*,*S*)-**4** (Figure 2).^[12]

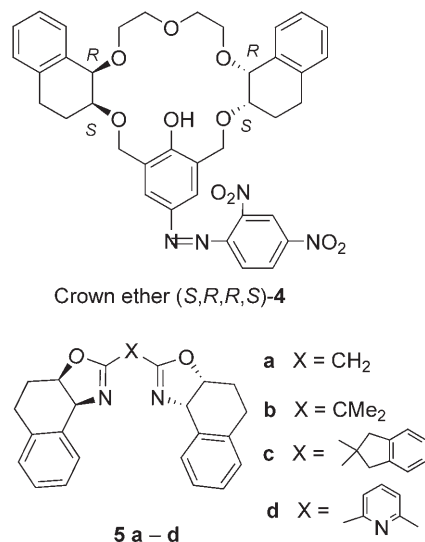


Figure 2.

A few biocatalytic methods have been reported for preparation of the optically active diol **1**. Boyd and co-workers have found that *Pseudomonas putida* UV4 and NCIMB 8859 oxidize 1,2-dihydronaphthalene to *cis*-(1*S*,2*R*)-diol **1** as a minor metabolite (*ca.* 21 % relative yield, >98 % *ee*) and to the *cis*-(1*R*,2*S*)-diol **1** antipode as a major metabolite (*ca.* 30 % relative yield, >98 % *ee*).^[13,14] Boyd also reported the formation of *trans*-(1*R*,2*R*)-diol **2** as the major biotransformation product of 1,2-dihydronaphthalene by rat liver microsomes in 75 % (54 % *ee*) and 60 % (4 % *ee*) relative yields.^[15]

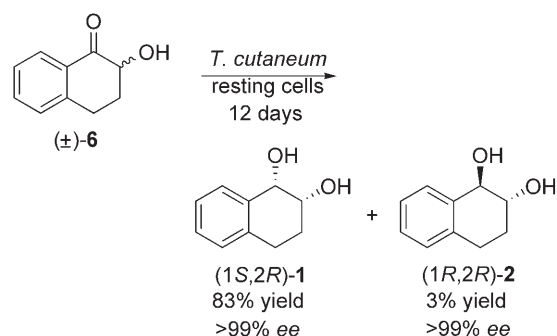
Gibson et al. have shown that *m*-xylene-induced cells of *Sphingomonas yanoikuyae* strains B1 and B8/36 were able to oxidize 1,2-dihydronaphthalene after 20–24 h to *cis*-(1*R*,2*S*)-diol **1** as the major products in 73 % and 44 % relative yields (>95 % *ee*), respectively.^[16] Also, Gibson found that the toluene dioxygenase of *Pseudomonas putida* F39/D oxidizes 1,2-dihydronaphthalene to *cis*-(1*S*,2*R*)-diol **1** and, in contrast, that the naphthalene dioxygenase of *Pseudomonas* sp. strain NCIB 9816/11 oxidizes the same substrate to the opposite enantiomer, *cis*-(1*R*,2*S*)-diol **1**.^[17] The *cis*-(1*S*,2*R*)-diol **1** was obtained in *ca.* 15 % relative yield while *cis*-(1*R*,2*S*)-diol **1** was obtained with *ca.* 70 % relative yield. Both compounds presented >98 % *ee*.

We have recently reported the preparation of (3*R*,4*S*)-3,4-chromanediol in high enantiomeric ex-

cesses (92–99 % *ee*) and reasonable isolated yields (38–58 %), through the deracemization of (±)-3-hydroxy-4-chromanone mediated by resting cells of *Trichosporon cutaneum* CCT 1903.^[18] We report herein the diastereo- and enantioselective preparation of (1*S*,2*R*)-1,2-dihydroxy-1,2,3,4-tetrahydronaphthalene (**1**) in high enantiomeric excess (>99 % *ee*) and excellent chemical yield (83 %) by the asymmetric reduction of racemic 2-hydroxy-1-tetralone (**6**) mediated by fresh resting cells of the yeast *Trichosporon cutaneum* through a DKR process.

Results and Discussion

The substrate (±)-**6** was prepared according to the methodology proposed by Moriarty, which is well established in the literature.^[19] Racemic 2-hydroxy-1-tetralone (**6**) was added to a slurry of resting cells of *T. cutaneum* (in distilled water). The yeast cells were previously cultivated in Sabouraud dextrose broth (SDB) for 3 days and then harvested by centrifugation. The reaction was monitored by periodic sampling of aliquots (1 mL), which were extracted with ethyl acetate and analyzed by GC/MS (Table 1). After incubation on a shaker (170 rpm) at 30 °C for 12 days, (±)-**6** was converted into a mixture of *cis*-(1*S*,2*R*)-diol **1** and (1*R*,2*R*)-*trans*-1,2-dihydroxy-1,2,3,4-tetrahydronaphthalene (**2**). The major product, *cis*-(1*S*,2*R*)-diol **1**, was obtained in 83 % isolated yield and high enantiomeric excess (>99 %) whilst the minor product, *trans*-(1*R*,2*R*)-diol **2**, was obtained in 3 % isolated yield and >99 % *ee* (Scheme 1).



Scheme 1.

During the course of the reaction, which was monitored by GC/MS analysis with a chiral column, the enantiomeric excess of the (*S*)-**6** increased during the first 68 h (Table 1, entry 4). This increase was due to the consumption of (*R*)-**6** of the (±)-2-hydroxy-1-tetralone (**6**), which was diastereo- and enantioselectively reduced to produce *cis*-(1*S*,2*R*)-diol **1**. The transformation is diastereoselective since the *cis*-**1** and *trans*-diols **2** were potentially expected products, but the

Table 1. Biotransformation of ($\pm$)-**6** by resting cells of *T. cutaneum* at 30 °C.

Entry	Time [h]	Yield [%] ^[a]		<i>trans</i> -diol 2 ^[c]	<i>ee</i> % [Configuration] ^[b]	
		6	<i>cis</i> -diol 1		6 ^[d]	<i>cis</i> -diol ^[e]
1	0	100	-	-	<i>racemate</i>	-
2	5	89	10	1	26 (<i>S</i>)	92 (1 <i>S</i> ,2 <i>R</i>)
3	20	70	29	1	69 (<i>S</i>)	83 (1 <i>S</i> ,2 <i>R</i>)
4	68	55	42	3	94 (<i>S</i>)	81 (1 <i>S</i> ,2 <i>R</i>)
5	116	42	57	1	45 (<i>S</i>)	88 (1 <i>S</i> ,2 <i>R</i>)
6	143	24	75	1	9 (<i>S</i>)	90 (1 <i>S</i> ,2 <i>R</i>)
7	169	14	85	1	46 (<i>R</i>)	96 (1 <i>S</i> ,2 <i>R</i>)
8	193	5	94	1	73 (<i>R</i>)	98 (1 <i>S</i> ,2 <i>R</i>)
9	288	6	93	1	96 (<i>R</i>)	> 99 (1 <i>S</i> ,2 <i>R</i>)

^[a] Chromatographic yield.^[b] Absolute configuration.^[c] *trans*-(1*R*,2*R*)-diol **2**.^[d] The *ee* of ($\pm$)-**6** was determined by GC/MS analysis using a Hydrodex- β 3P fused capillary chiral column.^[e] The *ee* of diol **1** was determined by HPLC using a chiral column Chiracel OJ-H, eluent hexane:2-propanol (90:10).

former was by far the major product. The reaction is considered to be enantioselective since although two stereoisomers are expected for the major product (*cis*-diol), only the *cis*-(1*S*,2*R*)-diol **1** was isolated as the sole product.^[20]

After the first 68 h of the reaction, (*R*)-**6** was almost entirely depleted and selectively converted into the *cis*-diol **1**. The remaining (*S*)-**6** was slowly converted into (*R*)-**6** until the end of the reaction. It is worth mentioning that after 143 h of reaction, the GC analysis indicated the formation of a racemic mixture of **6** and thereafter the enantiomeric excess of (*S*)-**6** decreased (Table 1, entry 6).

The formation of the products was followed by chiral HPLC analysis, which gave a high enantiomeric excess for the major *cis*-(1*S*,2*R*)-diol **1** during the course of the reaction (Table 1, entries 1–9). After 12 days of incubation, the experiment was stopped, the yeast cells were removed by centrifugation and the diols were recovered from the aqueous phase by extraction with ethyl acetate. After purification of the crude extract by flash column chromatography, *cis*-(1*S*,2*R*)-diol **1** was isolated in 83 % yield and > 99 % *ee* (Scheme 1). Then, (*R*)-**6** was recovered in 6 % yield with 96 % *ee*.

A yield greater than 50 % was achieved for diol **1**, and it is clear that *T. cutaneum* was able to promote deracemization of ($\pm$)-**6** through a DKR process. DKR effectively combines the resolution step of kinetic resolution with an *in situ* equilibration, or racemization, of the chirally labile substrate. As outlined previously,^[3b,c] DKR needs two supplementary steps to take place: racemization with a consecutive asymmetric transformation. Consequently, the faster reacting (*R*)-**6** is depleted during the course of the enantioselective reaction and the equilibrium of *R/S*-enantiomers is constantly re-adjusted by racemization of the slow-reacting counterpart (*S*)-**6**.

To explain the experimental data obtained for deracemization of ($\pm$)-2-hydroxy-1-tetralone (**6**), we reasoned that an isomerase is required to perform the isomerization of unreactive (*S*)-**6** during the biotransformation reaction.^[21] Thus, only (*R*)-**6** is reduced to the *cis*-diol **1**. In order to confirm these hypotheses, more detailed studies were conducted to elucidate the reaction mechanism proposed here. Experiments were carried out under the same experimental conditions described above, but this time racemic **6** was replaced by (*S*)- and (*R*)-2-hydroxy-1-tetralone (**6**) (96 % and 99 % *ee*, respectively) as substrates. The enantiomer (*R*)-**6** was prepared by the oxidation of the *cis*-(1*S*,2*R*)-diol **1** through a standard Jones' protocol. The antipode (*S*)-**6** was readily available through biocatalysis using the yeast *Pichia stipitis* (see Experimental Section).

For the experiments performed with (*R*)-**6**, the analysis of the chromatograms obtained after 24 h of reaction showed 76 % conversion to the *cis*-(1*S*,2*R*)-diol **1** and 18 % conversion to the *trans*-(1*R*,2*R*)-diol **2**. Total consumption of the substrate (*R*)-**6** was achieved after 30 h of incubation and the diols (1*S*,2*R*)-**1** and (1*R*,2*R*)-**2** were isolated in 60 % and 10 % yield, respectively, both with > 99 % *ee*. It is worth mentioning that no intermediates could be detected throughout the reaction; neither the isomerization of (*R*)-**6** to (*S*)-**6** nor the oxidation of (*R*)-**6** to 3,4-dihydro-1,2-naphthalenedione was observed.

On the other hand, the experiment performed with the (*S*)-**6** isomer demonstrated that its biotransformation is a sluggish process (Table 2). Therefore, after 24 h of incubation, only 23 % of (*S*)-**6** was converted to the *cis*-(1*S*,2*R*)-diol **1**, with 19 % *ee* (Table 2, entry 5) and the enantiomeric excess of (*S*)-**6** decreased to 81 %. In the course of the reaction, (*S*)-**6** was isomerized to the (*R*)-**6** isomer and then (*R*)-**6** was further reduced to *cis*-diol **1** until almost com-

Table 2. Biotransformation of (*S*)-**6** (96% *ee*) by resting cells of *T. cutaneum* at 30°C.

Entry	Time [h]	Yield [%] ^[c]			6 ^[a]	<i>ee</i> % ^[b] [Configuration] ^[d]	
		6	<i>cis</i> -diol 1	<i>trans</i> -diol 2		<i>cis</i> -diol 1	<i>trans</i> -diol 2
1	0	100	-	-	96 (<i>S</i>)	-	-
2	2	100	-	-	91 (<i>S</i>)	-	-
3	6	95	5	-	87 (<i>S</i>)	4 (1 <i>S</i> ,2 <i>R</i>)	-
4	18	77	19	4	83 (<i>S</i>)	10 (1 <i>S</i> ,2 <i>R</i>)	17 (1 <i>R</i> ,2 <i>R</i>)
5	24	73	23	4	81 (<i>S</i>)	19 (1 <i>S</i> ,2 <i>R</i>)	22 (1 <i>R</i> ,2 <i>R</i>)
6	48	62	34	5	79 (<i>S</i>)	38 (1 <i>S</i> ,2 <i>R</i>)	26 (1 <i>R</i> ,2 <i>R</i>)
7	72	45	50	5	73 (<i>S</i>)	56 (1 <i>S</i> ,2 <i>R</i>)	30 (1 <i>R</i> ,2 <i>R</i>)
8	96	29	68	5	61 (<i>S</i>)	67 (1 <i>S</i> ,2 <i>R</i>)	44 (1 <i>R</i> ,2 <i>R</i>)
9	144	9	86	5	28 (<i>S</i>)	83 (1 <i>S</i> ,2 <i>R</i>)	52 (1 <i>R</i> ,2 <i>R</i>)
10	288	5	89	6	16 (<i>S</i>)	> 99 (1 <i>S</i> ,2 <i>R</i>)	60 (1 <i>R</i> ,2 <i>R</i>)

^[a] The *ee* of (*S*)-**6**, determined by GC/MS analysis through a Hydrodex-β 3P fused capillary chiral column.

^[b] The *ee* of diol **1** and **2** was determined by HPLC using a chiral column Chiracel OJ-H, eluent hexane:2-propanol (90:10).

^[c] Chromatographic yield.

^[d] Absolute configuration.

plete consumption of the initial substrate. It is interesting to note that, although the *ee* of *cis*-(1*S*,2*R*)-diol **1** initially formed was low (4% *ee*, Table 2, entry 3), as the reaction progressed, it increased drastically.

It should be emphasized as already stated for the (*R*)-**6**, that no trace of side-products was found for the biotransformation of the (*S*)-**6** within the detection limits. Since intermediates like diones are absent, it is reasonable to believe that the isomerization of (*S*)-**6** is performed by enzymes from *T. cutaneum*.

After 12 days of incubation, the reaction was stopped and products were extracted and purified by flash column chromatography on silica gel. The diols *cis*-(1*S*,2*R*)-**1** and *trans*-(1*R*,2*R*)-**2** were isolated in 47% (> 99% *ee*) and 3% (60% *ee*) yields, respectively. In addition, substrate (*S*)-**6** was recovered in 20% yield and 16% *ee*.

To confirm that the isomerization of ketone (*S*)-**6** into the (*R*)-isomer was a complete enzymatic transformation and not a “spontaneous” process, experiments with (*S*)-**6** (96% *ee*) and (*R*)-**6** (99% *ee*) in the

absence of yeast cells were carried out under the same experimental conditions as already described for the deracemization of (±)-ketone **6**. After 10 days of incubation, no isomerization of either substrate was observed and starting materials were recovered in high yield. Thus, these data strongly suggest that the isomerization step is not “spontaneous” but should be attributed to specialized enzymes of *T. cutaneum*, such as isomerases, a class of enzymes able to perform biological inversions of stereochemistry.^[2a]

To elucidate the unusual formation of *cis*-(1*S*,2*R*)-diol **1** as the major product of the reduction of (*S*)-**6** and to understand the conversion of the (1*R*,2*S*)-diol **1** to the (1*S*,2*R*)-diol **1** (since the enantiomeric excess of the latter increases to > 99%), further experiments using the *cis*-(1*R*,2*S*)-diol **1** and *cis*-(1*S*,2*R*)-diol **1** as substrates (both > 99% *ee*), and *T. cutaneum* as the biocatalyst were carried out under the same standard experimental conditions as already described here. The results for the *cis*-(1*R*,2*S*)-diol **1** (see Experimental Section for its preparation) are shown in Table 3.

Table 3. Biotransformation of *cis*-(1*R*,2*S*)-diol **1** (> 99% *ee*) by resting cells of *T. cutaneum* at 30°C.

Entry	Time (days)	Yield [%] ^[c]			6 ^[a]	<i>ee</i> % ^[b] [Configuration] ^[d]	
		6	<i>cis</i> -diol 1	<i>trans</i> -diol 2		<i>cis</i> -diol 1	<i>trans</i> -diol 2
1	4 h	3	93	1	-	> 99 (1 <i>R</i> ,2 <i>S</i>)	> 99 (1 <i>R</i> ,2 <i>R</i>)
2	1	20	76	1	93 (<i>S</i>)	96 (1 <i>R</i> ,2 <i>S</i>)	> 99 (1 <i>R</i> ,2 <i>R</i>)
3	2	32	64	1	93 (<i>S</i>)	86 (1 <i>R</i> ,2 <i>S</i>)	> 99 (1 <i>R</i> ,2 <i>R</i>)
4	3	42	56	< 2	89 (<i>S</i>)	72 (1 <i>R</i> ,2 <i>S</i>)	> 99 (1 <i>R</i> ,2 <i>R</i>)
5	4	43	38	< 2	64 (<i>S</i>)	38 (1 <i>R</i> ,2 <i>S</i>)	> 99 (1 <i>R</i> ,2 <i>R</i>)
6	7	19	77	1	1 (<i>R</i>)	64 (1 <i>S</i> ,2 <i>R</i>)	> 99 (1 <i>R</i> ,2 <i>R</i>)
7	10	33	66	< 1	96 (<i>R</i>)	91 (1 <i>S</i> ,2 <i>R</i>)	> 99 (1 <i>R</i> ,2 <i>R</i>)
8	14	42	57	0	> 99 (<i>R</i>)	99 (1 <i>S</i> ,2 <i>R</i>)	-

^[a] The *ee* of (*S*)-**6**, determined by GC/MS analysis through a Hydrodex-β 3P fused capillary chiral column.

^[b] The *ee* of diol **1** and **2** was determined by HPLC using a chiral column Chiracel OJ-H, eluent hexane:2-propanol (90:10).

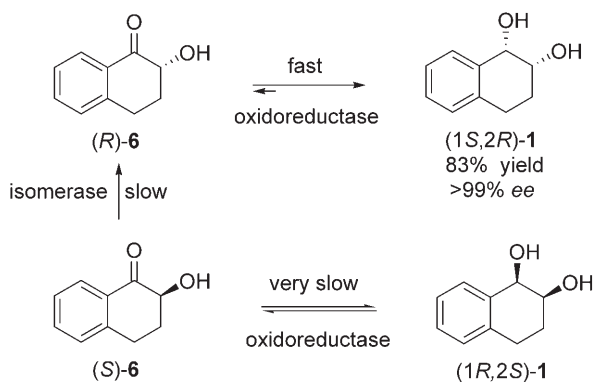
^[c] Chromatographic yield.

^[d] Absolute configuration.

With respect to the formation of (*S*)-ketone **6**, it is evident that the *cis*-(1*R*,2*S*)-diol **1** is enzymatically oxidized to (*S*)-ketone **6**, which is slowly isomerized to (*R*)-ketone **6**. Thereafter, the formation of the *cis*-(1*S*,2*R*)-diol **1** is a consequence of a further and faster reduction of (*R*)-ketone **6**, rather than (*S*)-ketone **6**, by the yeast cells. After 14 days of incubation, the stereoinversion of *cis*-(1*R*,2*S*)-diol **1** resulted in *cis*-(1*S*,2*R*)-diol **1** (40% yield) and (*R*)-**6** (22% yield), both in high enantiomeric excess (Table 3, entry 8). Furthermore, the experiment performed with the *cis*-(1*S*,2*R*)-diol **1** revealed that (*R*)-ketone **6** was the sole product observed, even after 14 days of incubation (6% yield, >99% *ee*).

The deracemization of ketone **6** by *T. cutaneum* was scaled up to a 0.5–1.0 g scale of the substrate without loss of the high isolated yield or of the high enantiomeric excess of the product. Hence, after 12 days of incubation, the diol (1*S*,2*R*)-**1** was isolated in 82% and 80% yield (0.5 and 1.0 g scales, respectively) and the unreacted substrate was typically recovered in 6% and 12% yield (0.5 g and 1.0 g scales, respectively).

The experiments performed with the chiral substrates show that the deracemization of the title compound is not a simple process. The formation of *cis*-(1*S*,2*R*)-diol **1** seems to require both the intervention of an isomerase to convert (*S*)-ketone **6** into (*R*)-ketone **6** and an equilibrium-controlled oxidation-reduction sequence to convert the *cis*-(1*R*,2*S*)-diol **1** back to the (*S*)-ketone **6** and keep the balance between diols and hydroxy ketones (Scheme 2). Con-



Scheme 2.

cerning the former transformations (from the point of view from the substrates) a typical DKR could be considered. A closer look at the latter transformations (from the point of view from the products) shows that the product diol *cis*-(1*R*,2*S*)-diol **1** is being ultimately converted to the *cis*-(1*S*,2*R*)-diol **1** through a stereoinversion-like process.

Conclusions

A biocatalytic process to deracemize ($\pm$)-2-hydroxy-1-tetralone (**6**) efficiently has been successfully devised using the versatile, easily-cultivated, non-conventional yeast *Trichosporon cutaneum* CCT 1903 to prepare the (1*S*,2*R*)-diol **1** in enantiomerically pure form (>99% *ee*) and in high yield (83%). In order to understand the mechanism of the complete dynamic kinetic resolution of the substrate, a rationale based on a comprehensive study with chiral substrates was proposed. In this mechanism, the intervention of isomerase and the oxidation-reduction equilibrium are key features to be highlighted. Moreover, the enzymatic DKR of ($\pm$)-**6** is unprecedented in the literature and presents a promising environmentally friendly method to obtain enantiopure (1*S*,2*R*)-diol **1** in larger scales.

Experimental Section

General Remarks

All reagents and solvents were obtained from commercial sources. Ethyl acetate, hexane, methanol and chloroform were distilled before use. ^1H and ^{13}C NMR spectra were recorded in CDCl_3 and DMSO using an INOVA-500 or Varian Gemini 300P spectrometer. Optical rotations were measured on a Perkin-Elmer Polarimeter 341. Melting points were measured on a Microquímica MQ APF-301. HPLC analyses were carried out using a Shimadzu LC-20 AT prominence instrument and a chiral column Chiralcel OJ-H from Daicel. GC/MS analysis and mass spectra were obtained on a QP 5000-Shimadzu or an Agilent CG 6890/Hewlett Packard 5973 equipped with HP-5MS (5% phenylmethylpolysiloxane, 30 m $\times$ 250 μm $\times$ 0.25 μm) and Macherey 212117/91 Hydrodex- β 3P fused capillary columns with either helium or hydrogen as carrier gas (1.0 mL min^{-1}). The strains of *T. cutaneum* (CCT 1903) and *Pichia stipitis* (CCT 2617) were purchased from the "André Tosello" Research and Technology Tropical Foundation (Brazil). Bactopeptone was purchased from Biobrás (Brazil). Thin layer chromatography (TLC) analyses were performed with pre-coated aluminum sheets (silica gel 60 Merck) and flash column chromatography was carried out on silica (200–400 mesh, Merck).

Synthesis of ($\pm$)-2-Hydroxy-1-tetralone (**6**)

The substrate ($\pm$)-**6** was prepared through hypervalent iodine oxidation according to Moriarty's protocol.^[19] Then, the commercially available α -tetralone (1.0 g; 6.85 mmol) was converted to 2-hydroxy-1-tetralone dimethyl ketal, isolated as a dark red oil; GC/MS: m/z (%) = 210 [M^+] (9), 178 (24), 166 (100), 150 (36), 134 (48), 120 (82), 119 (88), 107 (26), 91 (89), 77 (28), 63 (15), 51 (10).

The ketal was then submitted to hydrolysis with 3 mol L^{-1} aqueous HCl in ethanol for 1 hour. After neutralization with NaHCO_3 and extraction with ethyl acetate, purification of ($\pm$)-2-hydroxy-1-tetralone (**6**) was achieved by Kugelrohr

distillation utilizing a preheated oven (130°C) at 15 mmHg. Compound ($\pm$)-**6** was obtained as a colorless oil; yield: 0.98 g (6.05 mmol; 89%); IR (KBr): ν =3474, 1686, 1603, 1434, 1282, 1090, 994, 931, 757 cm^{-1} ; GC/MS: m/z (%) = 162 [M^+] (42), 144 (20), 133 (12), 118 (100), 116 (36), 105 (8), 90 (65), 77 (11), 63 (9), 51 (8); ^1H NMR (300 MHz, CDCl_3): δ =2.17–2.32 (m, 1H), 2.69–2.78 (m, 1H), 3.18–3.42 (m, 2H), 4.14 (s, 1H, OH), 4.59 (dd, J =13.5 and 5.7 Hz, 1H), 7.47 (d, J =7.8 Hz, 1H), 7.54 (t, J =7.5 Hz, H-1), 7.72 (td, J =7.5 and 1.5 Hz, 1H), 8.23 (d, J =8.1 Hz, 1H); ^{13}C NMR (75.5 MHz, CDCl_3): δ =27.7, 31.8, 73.8, 126.8, 127.5, 128.8, 130.4, 134.1, 144.3, 199.6.

Growth Conditions of *Trichosporon cutaneum* CCT 1903 and *Pichia stipitis* CCT 2617

Cells of *T. cutaneum* CCT 1903 were grown in sterile SDB (Sabouraud dextrose broth, 1 L) for 3 days at 30°C on an orbital shaker (170 rpm). The yeast *Pichia stipitis* CCT 2617 was cultivated in YM (yeast-malt extract) nutrient broth (3 L) for 2 days at 28°C on an orbital shaker (170 rpm). The cells were harvested by centrifugation (5000 rpm, 10 min) prior to use in the reactions.

General Procedure for Biotransformation

Biotransformation of ($\pm$)-2-hydroxy-1-tetralone (6**):** A solution of ($\pm$)-2-hydroxy-1-tetralone (**6**) (250 mg) in ethanol (2.5 mL) was added to a slurry of *T. cutaneum* CCT 1903 (15 g, wet weight) in glucose (5 g) and sterile distilled water (250 mL). The resulting suspension was stirred on an orbital shaker (170 rpm) at 30°C until total consumption of **6** (ca. 12 days). After centrifugation (5000 rpm), the supernatant and cell mass were thoroughly extracted with ethyl acetate. The organic extracts were combined, dried over anhydrous Na_2SO_4 , and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography on silica gel (hexane:ethyl acetate, 1:1) to give *cis*-(1*S*,2*R*)-diol **1** (yield: 210 mg, 1.28 mmol, 83%, >99% *ee*) and *trans*-(1*R*,2*R*)-diol **2** (yield: 7.6 mg, 0.046 mmol, 3%, >99% *ee*). A small amount of substrate was recovered (15 mg, 6%) and showed 96% *ee* for (*R*)-**6**. The absolute configurations were assigned by comparison of the sign of the specific rotation with that reported in the literature.^[13]

Data for (1*S*,2*R*)-1**:** white crystals, mp 135–136°C (Lit.^[13] 129–130°C); $[\alpha]_{\text{D}}^{20}$: +33.6° (c 3.1, CHCl_3) [Lit.^[13] $[\alpha]_{\text{D}}^{20}$: +39° (c 3.1, CHCl_3) for >98% *ee*]; ^1H NMR (300 MHz, DMSO): δ =1.66–1.77 (m, 1H), 1.86–1.98 (m, 1H), 2.60–2.70 (m, 1H), 2.80–2.90 (m, 1H), 3.77–3.84 (m, 1H), 4.43–4.47 (m, 1H), 4.51 (d, J =4.8 Hz, 1H), 4.84 (d, J =5.7 Hz, 1H), 7.03–7.08 (m, 1H), 7.12–7.15 (m, 2H), 7.32–7.35 (m, 1H); ^{13}C NMR (75.5 MHz, DMSO): δ =26.0, 26.3, 68.3, 69.2, 125.2, 126.6, 127.6, 129.1, 135.7, 138.3; IR (KBr): ν =3259, 2935, 1459, 1071, 966; 775, 738 cm^{-1} ; GC/MS: m/z = 164 (M^+ , 11%), 146 (54%), 120 (95%), 119 (100%), 91 (46%), 77 (11%), 65 (11%), 51 (6%).

Data for (1*R*,2*R*)-2**:** white crystals, $[\alpha]_{\text{D}}^{20}$: +70.5° (c 0.31, CHCl_3) [Lit.^[22] data for enantiomer (1*S*,2*S*)-**2**; $[\alpha]_{\text{D}}^{20}$: –110° (CHCl_3)]; ^1H NMR (300 MHz, DMSO): δ =1.61–1.74 (m, 1H), 1.90–2.10 (m, 1H), 2.64–2.86 (m, 2H), 3.62–3.72 (m, 1H), 4.27 (t, J =5.85, 1H), 4.82 (d, J =3.9 Hz, 1H), 5.25 (d, J =5.4 Hz, 1H), 7.02–7.06 (m, 1H), 7.11–7.16 (m, 2H), 7.37–7.41 (m, 1H); GC/MS: m/z = 164 (M^+ , 11%), 146

(53%), 120 (95%), 119 (100%), 91 (45%), 77 (11%), 65 (11%), 51 (6%).

The biotransformation of ($\pm$)-**6** was carried out for 12 days on 0.5 g (3.08 mmol) and 1.0 g (6.17 mmol) scales, under the same experimental conditions as already described above. The product was extracted into ethyl acetate, dried and concentrated. After purification by flash column chromatography on silica gel (hexane:ethyl acetate, 2:1 and 1:1), *cis*-(1*S*,2*R*)-diol **1** (>99% *ee*) was isolated in 82% (415 mg, 2.53 mmol) and 80% (810 mg, 4.94 mmol) yields, respectively. The *trans*-(1*R*,2*R*)-diol **2** (>99% *ee*) was also obtained in 3% isolated yield for both experiments. The substrate **6** was recovered in 6% and 12% yields (0.5 g and 1.0 g scale, respectively).

Biotransformation of (*R*)-2-hydroxy-1-tetralone (6**):** The biotransformation of (*R*)-**6** (45 mg, 0.28 mmol, 99% *ee*) was carried out under the same experimental conditions as already described above. After 30 h the product was extracted into ethyl acetate, dried and evaporated. Purification by flash column chromatography on silica gel (hexane:ethyl acetate, 1:1) gave *cis*-(1*S*,2*R*)-diol **1** ($[\alpha]_{\text{D}}^{20}$: +30.6° (c 2.0, CHCl_3), >99% *ee*) and *trans*-(1*R*,2*R*)-diol **2** (>99% *ee*) in 60% (27 mg, 0.17 mmol) and 10% (5 mg, 0.028 mmol) yields, respectively.

Biotransformation of (*S*)-2-hydroxy-1-tetralone (6**):** (*S*)-**6** (50 mg, 0.31 mmol, 96% *ee*) was biotransformed as describe before. After 12 days of incubation, the products were extracted and purified by flash column chromatography on silica gel. The *cis*-(1*S*,2*R*)-diol **1** ($[\alpha]_{\text{D}}^{20}$: +31.67° (c 2.25, CHCl_3), >99% *ee*) and *trans*-(1*R*,2*R*)-diol **2** (60% *ee*) were isolated in 47% (24 mg, 0.145 mmol) and 3% (1.5 mg, 0.009 mmol) yields, respectively. The substrate (*S*)-**6** was recovered in 20% yield and 16% *ee*.

Biotransformation of *cis*-(1*S*,2*R*)- and *cis*-(1*R*,2*S*)-1,2-dihydroxy-1,2,3,4-tetrahydronaphthalene (1**):** The *cis*-(1*S*,2*R*)- and *cis*-(1*R*,2*S*)-diol **1** (50 mg each compound) were also used as substrate in the same experimental conditions as described above. The reaction mixture was incubated at 30°C in an orbital shaker (170 rpm) for 14 days. The cells were then separated and the products extracted into ethyl acetate, concentrated and purified by flash column chromatography on silica gel. In the experiments, *cis*-(1*S*,2*R*)-diol **1** (99% *ee*) was isolated in 76% and 40% yield, respectively. The oxidation product (*R*)-**6** (99% *ee*) was also obtained in 6% and 22% yield, respectively.

Preparation of (*R*)-2-Hydroxy-1-tetralone (**6**)

To a stirred solution of (1*S*,2*R*)-diol **1** (2.0 g, 0.012 mol) in acetone (40 mL) at 0°C, Jones' reagent (10 mL) was added dropwise. After complete addition of the oxidizing agent, the mixture was stirred until the reaction reached completion. Methanol (20 mL) was added to quench excess of Jones' reagent. The reaction mixture was treated with a saturated aqueous solution of NaHCO_3 and extracted with ethyl acetate (4×30 mL). The organic phase was washed with a saturated aqueous solution of NaCl (3×50 mL), dried over Na_2SO_4 and concentrated at reduced pressure. The residue was purified by flash column chromatography on silica gel (hexane:ethyl acetate, 2:1) to give (*R*)-2-hydroxy-1-tetralone (**6**) as a yellow oil; yield: 395 mg (2.44 mmol, 20%);

$[\alpha]_{\text{D}}^{20}$: +44.5° (c 1.54, CHCl₃) with 99% *ee* {Lit.^[21a] $[\alpha]_{\text{D}}^{20}$: +50.0° (c 1.0, CHCl₃) for >99% *ee*}.

Preparation of (S)-2-Hydroxy-1-tetralone (2) and cis-(1R,2S)-diol 1

To a slurry of *Pichia stipitis* CCT 2617 (30 g, wet weight) and glucose (5 g), in sterile distilled water (0.8 L), a solution of racemic (±)-2-hydroxy-1-tetralone (6) (0.8 g) in ethanol (5 mL) was added. The resulting suspension was stirred on an orbital shaker (170 rpm) at 30°C for 48 h. After centrifugation (5000 rpm), the supernatant and cell mass were thoroughly extracted with ethyl acetate. The organic extracts were combined, dried over anhydrous Na₂SO₄, and the solvent was removed under reduced pressure. Purification was achieved by flash column chromatography on silica gel (hexane:ethyl acetate, 2:1 and 1:1) to furnish (S)-6 (yield: 0.54 g, 68%) and cis-(1R,2S)-diol 1 (yield: 0.15 g; 18%).

(S)-6: white crystals, mp 44.5–45.5°C, $[\alpha]_{\text{D}}^{20}$: –43.6° (c 1.57, CHCl₃) with 96% *ee*.

cis-(1R,2S)-diol 1: white crystals, mp 132–133.4°C, (Lit.^[23] 129–130°C); $[\alpha]_{\text{D}}^{20}$: –31.0° (c 0.89, CHCl₃) with >99% *ee* {Lit.^[23] $[\alpha]_{\text{D}}^{20}$: –38° (c 0.87, CHCl₃)}.

Synthesis of cis-(±)-1 and trans-(±)-1,2-Dihydroxy-1,2,3,4-tetrahydronaphthalene (2)

Compound (±)-6 (100 mg, 0.62 mmol) was reacted with sodium borohydride (30 mg, 0.79 mmol) in methanol (7 mL) at 0–5°C to result in a mixture of cis-1 and trans-diols 2 (1:9) after usual work-up. The products were separated by flash column chromatography on silica gel using hexane:ethyl acetate (1:1) as eluent to give (±)-1 (10 mg, 0.06 mmol, 10% yield) and (±)-2 (86 mg, 0.52 mmol, 85% yield), both as white crystals.

Determination of Enantiomeric Excess of the Substrate and Products Formed

The enantiomeric excesses of products 1 and 2 from the biotransformation reactions were analyzed on HPLC using a chiral column Chiracel OJ-H from Daicel, Japan. The mobile phase used was hexane:2-propanol (9:1), flow rate 0.7 mL min^{–1}, monitored at 265 nm. For comparison, racemic cis- and trans-diols standards were used. Retention times for the two diols were: cis-diols 1 (14.5 and 21.4 min) and trans-diols 2 (11.9 and 16.5 min). The *ee* of substrate (±)-6 was determined by GC/MS analysis through a Hydrodex-β 3P fused capillary chiral column (25 m × 0.25 mm × 0.25 μm) using hydrogen as carrier gas. In this method the injector and the detector were set at 200°C and 230°C, respectively, initial column temperature was 50°C (3.0 min) increasing the temperature by 2.0°C min^{–1} until 180°C (1.0 min); retention times: (R)-6 at 32.2 min and (S)-6 at 32.4 min.

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