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Deracemization of racemic 1,2-diol by biocatalytic stereoinversion

Animesh Goswami,* K. David Mirfakhrae and Ramesh N. Patel

Enzyme Technology, Process Research and Development, Bristol-Myers Squibb Pharmaceutical Research Institute, New Brunswick, NJ 08903, USA

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Abstract

Deracemization of racemic 1,2-diol, (*RS*)-1-{2′,3′-dihydrobenzo[*b*]furan-4′-yl}-ethane-1,2-diol (**1**), by stereoinversion by several microorganisms belonging to the genera *Candida* and *Pichia* provided the corresponding chiral (*S*)-diol, (*S*)-1-{2′,3′-dihydrobenzo[*b*]furan-4′-yl}-ethane-1,2-diol (**2**). (*S*)-Diol **2** was obtained in yields of 60–70% with 90–100% enantiomeric excess.

1. Introduction

One of the most used techniques for the development of chiral compounds involves biocatalytic resolution. Though deracemization by kinetic resolution often provides compounds with high enantiomeric excess, the maximum theoretical yield of product or substrate is only 50% in such a process.

This problem of kinetic resolution can be solved by employing a deracemization technique known as stereoinversion. In stereoinversion, one enantiomer of the alcohol is oxidized to the ketone while the other enantiomer of the alcohol remains unchanged. The ketone is reduced to the opposite enantiomer of the alcohol during the subsequent reduction process. The net result is the conversion of the racemic alcohol to a single enantiomer of the alcohol in potentially 100% yield. Stereoinversion thus overcomes the limitation of the maximum theoretical yield of 50% encountered during kinetic resolution of alcohol with enzymes.

Only a handful of reports appeared in the recent literature on the deracemization of alcohols by dynamic resolution and stereoinversion.[1–12] *Geotrichum candidum*, *Candida parapsilosis* and a few other species are reported to be effective in such processes. Dynamic resolutions with a biocatalyst and metal catalyzed in situ racemizations have also been reported.[13,14]

* Corresponding author. E-mail: animesh.goswami@bms.com

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Table 1
Summary of screening microorganisms for deracemization of (*RS*)-diol **1** by stereoinversion

Microorganism	Strain ID	Time Days	EE (*S*) -Diol 2 %
Candida boidinii	ATCC 56507	6	100%
Candida boidinii	ATCC 26175	7	100%
Candida boidinii	ATCC 32195	7	93%
Pichia methanolica	ATCC 58403	7	90%
Pichia methanolica	ATCC 56508	7	69%
Pichia methanolica	ATCC 56510	7	87%
Pichia pinus	ATCC 28780	7	63%
Hansenula polymorpha	ATCC 66057	7	96%
Hansenula polymorpha	ATCC 62809	7	92%
Mortierella ramanniana	ATCC 34194	7	42%
Mortierella ramanniana	ATCC 24786	6	45%
Arthrobacter simplex	SC 6379	7	-33%
Candida parapsilosis	ATCC 52820	6	-20%

The present work describes for the first time the deracemization of (*RS*)-1-{2′,3′-dihydrobenzo[*b*]furan-4′-yl}-ethane-1,2-diol (**1**) to the (*S*)-diol, (*S*)-1-{2′,3′-dihydrobenzo[b]furan-4′-yl}-ethane-1,2-diol (**2**), by biocatalytic stereoinversion.

2. Results and discussion

Seven cultures were selected from the screening of 20 microorganisms as the leading candidates for stereoinversion. These were *Candida boidinii* ATCC 26175, ATCC 32195, ATCC 56507, *Pichia methanolica* ATCC 58403, ATCC 56510, and *Hansenula polymorpha* ATCC 66057, ATCC 62809. The relative proportions of (*S*)-diol **2** increased with time in the biotransformations with the above cultures. At the end of one week, the enantiomeric excess (ee) of the remaining (*S*)-diol **2** was found to be in the range of 87–100% with these microorganisms. The results are shown in Table 1. Only two microorganisms, *Candida parapsilosis* ATCC 52820 and *Arthrobacter simplex* SC 6379, showed a higher amount of (*R*)-diol. Interestingly, *Candida parapsilosis* was reported to be useful for stereoinversion of other diols by Hagesawa et al.[11]

A new compound was formed during these biotransformations as seen by the appearance of a new peak in the HPLC of the reaction mixtures; for example, the extracts from biotransfomations showed a new peak at 17 min on the reversed phase C-8 HPLC system. The (*RS*)-diol **1** eluted at 11 min in the same system. The new compound showed a mass of 178 in LC-MS. The starting (*RS*)-diol **1** showed the mass peak at 180 by LC-MS. The area of the HPLC peak for this new compound initially increased with time, reached a maximum, and then decreased. The hydroxy ketone **4** structure is tentatively proposed for this new compound based on LC-MS analysis. This would be consistent from the proposed pathway of stereoinversion (Fig. 1) in which the hydroxy ketone **4** is first formed by oxidation of the (*R*)-diol **3** and then subsequently reduced back to diol but only to the (*S*)-diol **2**.

The yield and ee of the diol at various times were followed for the transformation of (*RS*)-diol **1** by the seven microorganisms described above. The reactions were also conducted with and without glucose to investigate the effect of glucose on the course of the biotransformation. A summary of results is shown in Table 2.

Candida boidinii ATCC 32195, *Candida boidinii* ATCC 56507, and *Pichia methanolica* ATCC 56510 transformed the (*RS*)-diol **1** in 3–4 days and the (*S*)-diol **2** was obtained in 62–71% yield and 90–100% ee. It was also found to be important to stop the reaction at the highest yield and desired ee, as running

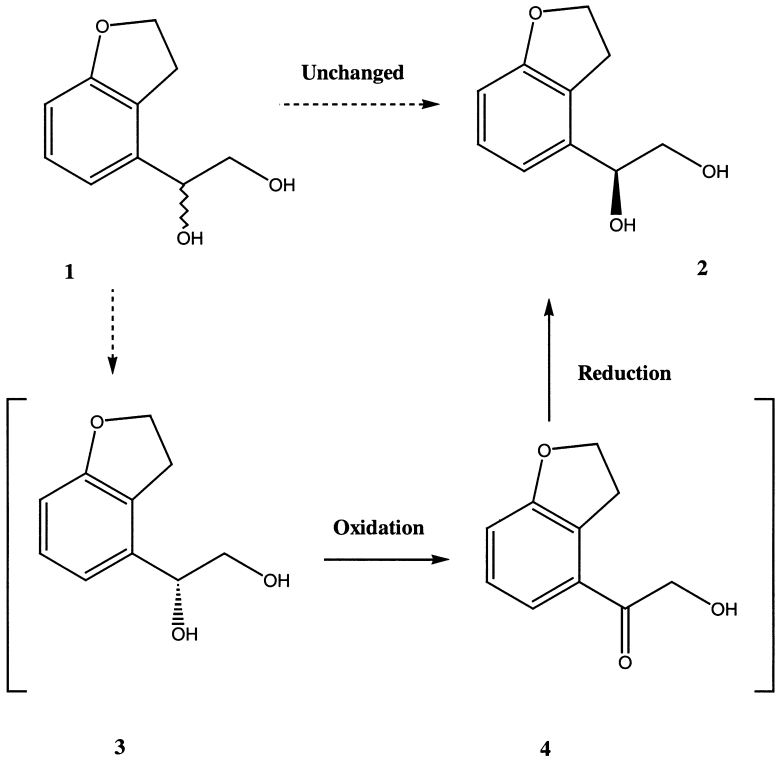

Figure 1. Deracemization of *RS*-diol **1** by stereoinversion

the reaction for a longer time decreased the yield. In a few cases, e.g. *Pichia methanolica* ATCC 58403 and *Hansenula polymorpha* ATCC 62809, the yields were high (~70%) but the ees were low (52–87%). Though some stereoinversion may be occurring in these cases, resulting in increased proportion of the (*S*)-diol **2**, the ee was low even after 3–4 days.

The yield was generally higher and the ee was lower when reactions were carried out in the presence of glucose. However, there were some exceptions. Glucose analysis at the end of the reaction showed the absence of glucose in all biotransformations. Glucose added during each day was probably consumed by the microbes.

3. Experimental

3.1. Chemicals

(*RS*)-Diol **1** and (*S*)-diol **2** were prepared by hydrolysis (as described below) of the corresponding epoxides.[15] The structural identity and purity of each compound was established by spectroscopic and other physical and chemical methods. Other chemicals were purchased from VWR and/or Aldrich.

3.2. Microorganisms

Microorganisms were obtained from the ATCC and BMS culture collection. The SC number denotes the number in the BMS culture collection.

Table 2
Detailed investigation of deracemization of *RS*-diol **1** by stereoinversion with seven microorganisms

Microorganism	ATCC #	Medium	Time Day	Remaining Diol %	EE (*S*) -Diol 2 %
Candida boidinii	26175	Buffer Only	4	70%	87%
Candida boidinii	26175	Buffer + Glucose	4	74%	54%
Candida boidinii	32195	Buffer Only	2	66%	57%
Candida boidinii	32195	Buffer Only	3	60%	91%
Candida boidinii	32195	Buffer Only	4	66%	90%
Candida boidinii	32195	Buffer + Glucose	2	64%	58%
Candida boidinii	32195	Buffer + Glucose	3	72%	84%
Candida boidinii	32195	Buffer + Glucose	4	62%	100%
Candida boidinii	56507	Buffer Only	2	58%	87%
Candida boidinii	56507	Buffer Only	3	74%	95%
Candida boidinii	56507	Buffer Only	4	64%	100%
Candida boidinii	56507	Buffer + Glucose	2	67%	80%
Candida boidinii	56507	Buffer + Glucose	3	71%	94%
Pichia methanolica	58403	Buffer Only	4	83%	63%
Pichia methanolica	58403	Buffer + Glucose	3	88%	54%
Pichia methanolica	58403	Buffer + Glucose	4	72%	87%
Pichia methanolica	56510	Buffer Only	2	52%	86%
Pichia methanolica	56510	Buffer Only	3	65%	100%
Pichia methanolica	56510	Buffer Only	4	46%	100%
Pichia methanolica	56510	Buffer + Glucose	2	57%	89%
Pichia methanolica	56510	Buffer + Glucose	3	67%	100%
Hansenula polymorpha	66057	Buffer Only	4	84%	44%
Hansenula polymorpha	66057	Buffer + Glucose	3	103%	32%
Hansenula polymorpha	62809	Buffer Only	4	73%	60%
Hansenula polymorpha	62809	Buffer + Glucose	3	74%	52%

3.3. Analytical methods

NMR spectra were recorded in DMSO solution on a Varian 500 MHz NMR spectrophotometer operating at 499.7 MHz for ^{1}H and 125.6 MHz for ^{13}C.

The HPLC analysis of the diol was done on the following systems:

Reversed phase C-8 column (Kromasil, 15 cm×0.46 cm) at ambient temperature using a gradient elution starting from water to acetonitrile:water (50:50) in 30 min at a flow rate of 1 ml/min and detection by UV at 210 nm. The (*RS*)-diol **1** eluted at 11 min.

Pirkle Covalent SS Whelk column (25 cm×0.46 cm) at ambient temperature with hexane:isopropanol (95:5) at 1.2 ml/min and detection by UV at 210 nm. (*S*)- and (*R*)-Diols eluted at 21.2 min and 22.4 min, respectively, and their areas were used to determine their ees.

3.4. Hydrolysis (RS)-epoxide: preparation of (RS)-diol *1*

(*RS*)-1-{2′,3′-Dihydrobenzo[*b*]furan-4′-yl}-1,2-oxirane[15] (1 g) was added to 100 mM phosphate buffer (pH 5) and the mixture was stirred at room temperature for 48 h. The pH was adjusted to 7 and NaCl (100 g) was added. The aqueous solution was extracted with ethyl acetate (3×300 ml). The organic layer was dried over anhydrous Na_2SO_4 and filtered. After removal of ethyl acetate, the residue

was crystallized from a mixture of heptane and ethyl acetate to provide (*RS*)-diol **1**; 731 mg; LC-MS: M^+ 180; ^{1}H NMR: δ 3.17 (m, 2H), 3.42 (m, 2H), 4.48 (m, 2H), 4.53 (t, 1H, J=6 Hz), 4.74 (br s, 1H), 5.2 (br s, 1H), 6.62 (d, 1H, J=7.7 Hz), 6.83 (d, 1H, J=7.7 Hz), 7.03 (t, 1H, J=7.7 Hz); ^{13}C NMR: δ 28.14, 66.21, 70.69, 72.47, 107.29, 117.99, 124.69, 127.41, 140.22, 159.39.

3.5. Growth of microorganisms

The medium for growing microorganisms was made as follows. Malt extract 10 g, yeast extract 10 g, peptone 1 g, dextrose 20 g were dissolved in distilled water to a total volume of 1 l, adjusted to pH 7.0 and autoclaved at 121°C for 20 min.

Sterilized medium (100 ml in a 500 ml flask) was inoculated with microorganisms from vials and allowed to grow by shaking at 200 rpm at 28°C for 72 h. The cells were harvested by centrifugation (10000 g for 20 min).

3.6. Procedure for screening microorganisms for deracemization of RS-*diol **1** by stereoinversion*

The cells (3 g) were suspended in 10 ml of 100 mM phosphate buffer (pH 7.0) in a 50 ml Erlenmeyer flask. To the cell suspension, a solution of 10 mg of (*RS*)-diol **1** in 50 μl DMF was added. The biotransformation was conducted by shaking at 200 rpm at 28°C. At various times, 1 ml of sample was withdrawn and extracted with 2 ml of ethyl acetate. The ethyl acetate layer was dried over anhydrous $MgSO_4$, filtered and evaporated to dryness under a stream of nitrogen, and the residue was analyzed by HPLC. At the end, the whole reaction mixture was extracted with ethyl acetate (double the volume of the remaining aqueous reaction mixture) and analyzed by HPLC.

3.7. Detailed study of the deracemization of RS-*diol **1** by stereoinversion with seven microorganisms*

The biotransformations were carried out as described above, using a solution of 5 mg of (*RS*)-diol **1** in 20 μl DMF. For reactions with glucose, 20 mg of glucose was added at the beginning of the experiment. Each day samples were withdrawn from each flask, extracted and analyzed by the methods described above to determine the ee and the amount of remaining diol. To the glucose reactions, a 50 μl aqueous solution containing 20 mg of glucose was added after each sample withdrawal to provide a fresh supply of glucose.

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