Stereoselective Enzymatic Reduction of 1,4-Diaryl-1,4-Diones to the Corresponding Diols Employing Alcohol Dehydrogenases

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Abstract: Due to the steric hindrance of the starting prochiral ketones, the preparation of chiral 1,4-diaryl-1,4-diols through the asymmetric hydrogen transfer reaction has been mainly restricted to the use of metal-based catalysts, oxazaborolidines, or organocatalysts. Herein, we demonstrated the versatility of oxidoreductases, finding overexpressed alcohol dehydrogenase from Ralstonia sp. (E. coli/RasADH) as the most active and stereoselective biocatalyst. Thus, the preparation of a set of 1,4-diaryl-1,4-diols bearing different pattern substitutions in the aromatic ring was achieved with complete diastereo- and enantioselectivity under mild reaction conditions.

Keywords: alcohol dehydrogenases; asymmetric synthesis; bioreduction; 1,4-diols; diketones

1. Introduction

Optically active 1,4-diols can be found as structural motifs in a huge number of biologically active compounds as pharmaceuticals, flavors, and fragrances, but also in chiral ligands and auxiliaries for asymmetric synthesis purposes [1,2]. Their synthetic versatility has also been utilized as precursors of valuable compounds such as 2,5-disubstituted pyrrolidines and phosphine derivatives used as ligands in asymmetric hydrogenations [3–5]. In this context, 1,4-butanediol plays a key role due its applications in the food and cosmetic industry, but also as precursor of plastics, pharmaceuticals, fibers, solvents, and biologically active lactones, among others. For these reasons, the preparation of enantioenriched 1,4-diols using efficient and selective methodologies is a field of interest in organic chemistry. Different asymmetric strategies have been reported for their synthesis, the catalytic asymmetric transfer hydrogenation (CATH) of prochiral 1,4-dicarbonylic compounds being one of the most recurrent methods [6,7]. CATH is the most straightforward approach for obtaining the optically pure diols in quantitative yield, but presents difficulty in that the hydroxy ketone intermediate can also be attained and that (up to) four possible diastereoisomers can be formed. Therefore, several selective chiral catalysts and reducing reagents have been developed for the stereoselective asymmetric reduction of these prochiral diketones, including rhodium or iridium complexes [8], different borane complexes such as oxazaborolidines [9,10], or proline-type derivatives as catalysts in combination with the appropriate reducing agents [11,12].

Biocatalysis has emerged in the last decades as a mature technology for the production of optically active (poly)alcohols by means of the stereoselection displayed by different enzymes [13–17].
Among the wide set of (poly)alcohol structures that can be obtained using biocatalysts, the production of optically active 1,4-dialkyl-1,4-diols and their corresponding acetates has been described mainly by the action of lipases through resolution procedures involving acylation and transesterification processes [18–25], and only the resolution of the bulkier 1-phenylpentane-1,4-diol or its acetate has been successfully achieved [26,27]. Alcohol dehydrogenases (ADHs) are valuable biocatalysts that have been widely employed for the synthesis of chiral alcohols due to their usually excellent selectivity [28–31], including dicarbonyl bioreduction processes [32,33]. Among the wide set of alcohol structures that can be obtained with these enzymes, optically active 1,4-dialkyl-1,4-diols have been described. Efforts have been especially focused on the preparation of chiral 2,5-hexanediol, a key starting material for the preparation of several catalysts and drugs [34–37]. However, until now there are no examples about the enzymatic reduction of 1,4-diaryl-1,4-diketones to obtain the corresponding chiral 1,4-diaryl-1,4-diols, as these substrates are probably too bulky for ADH recognition. In the present paper, we describe for the first time the use of ADHs for the stereoselective preparation under mild reaction conditions (aqueous buffer and 30 °C) of a representative panel of 1,4-diaryl-1,4-diols bearing different pattern substitutions in the aromatic ring.

2. Results and Discussion

As a first step, the chemical synthesis of prochiral 1,4-diketones 1–8a was developed to later study the biocatalyzed stereoselective synthesis of the optically active 1,4-diols 1–8b. Different methods have been described for the preparation of these substrates [38,39]. Among them, we have carried out the synthesis of these compounds through the cross aldol condensation of 4′-substituted acetophenones with α-bromo-4′-substituted acetophenones, followed by 1,3-dehydrobromination of these products, and cleavage of the corresponding activated cyclopropyl intermediates in the presence of ZnCl₂, t-BuOH, and Et₂NH (Scheme 1) [40]. Thus, 1,4-diketones 1–8a were obtained in yields varying from 8% for the o-methoxy derivative (8a) to 58% for 1,4-diphenylbutane-1,4-dione (1a).

Scheme 1. Synthesis of diketones 1–8a from the corresponding 4′-substituted acetophenones and the α-bromo-4′-substituted acetophenones.

Once synthesized, the obtained diketones were tested in the bioreduction processes catalyzed by a set of commercially available and “made in house” alcohol dehydrogenases. As model substrate 1,4-diphenylbutane-1,4-dione (1a) was chosen. Thus, lyophilized cells of E. coli overexpressing alcohol dehydrogenases from Ralstonia sp. (E. coli/RasADH) [41], Lactobacillus brevis (E. coli/LBADH) [42], Sphingobium yanoikuyae (E. coli/SyADH) [43], Thermoanaerobacter ethanolicus (E. coli/TeSADH) [44] and Rhodococcus ruber (E. coli/ADH-A) [45] were tested. From previous studies, 25 mM substrate concentration was initially considered and 2.5% v/v of dimethylsulfoxide (DMSO) was used as cosolvent due to the low solubility of the ketones in the aqueous media, using Tris-HCl buffer 50 mM pH 7.5, and isoamyl alcohol (IPA) or glucose dehydrogenase (GDH) with glucose as reducing agents to recycle the nicotinamide cofactor [46]. The reactions were incubated for 24 h at 30 °C, observing only activity when E. coli/RasADH was utilized (Table 1). This result is not entirely surprising, as this NADP-dependent enzyme has been previously described as a valuable biocatalyst for the reduction
of sterically hindered substrates [47]. Hence, a conversion of 56% was reached by using this enzyme, leading to the formation of diol (1S,4S)-1b in a highly selective manner (98% de, >99% ee), not observing the hydroxy ketone intermediate (Table 1, entry 1). Remarkably, 82% conversion was obtained after 48 h maintaining the excellent selectivity (Table 1, entry 2).

In order to optimize the bioreduction using this enzymatic preparation, different conditions were studied (Table 1, entries 3–17). Firstly, in similar conditions the absence of cosolvent led to a decrease in the conversion (72%–82% conversion, entries 2–3). When the usual regeneration system (glucose/GDH) was changed to IPA (entry 4), the diastereoselectivity of the process dropped from 98% de to 78% de.

Table 1. Bioreduction of 1,4-diphenylbutane-1,4-dione (1a) using E. coli/RasADH a.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Regeneration System</th>
<th>Cosolvent (v/v)</th>
<th>t (h)</th>
<th>Conversion (%) b</th>
<th>de (%) c</th>
<th>ee (%) d</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glucose/GDH</td>
<td>DMSO 2.5</td>
<td>24</td>
<td>56</td>
<td>99:1</td>
<td>&gt;99</td>
</tr>
<tr>
<td>2</td>
<td>Glucose/GDH</td>
<td>DMSO 2.5</td>
<td>48</td>
<td>82</td>
<td>99:1</td>
<td>&gt;99</td>
</tr>
<tr>
<td>3</td>
<td>Glucose/GDH</td>
<td>-</td>
<td>48</td>
<td>72</td>
<td>99:1</td>
<td>&gt;99</td>
</tr>
<tr>
<td>4</td>
<td>IPA</td>
<td>-</td>
<td>48</td>
<td>72</td>
<td>89:11</td>
<td>&gt;99</td>
</tr>
<tr>
<td>5</td>
<td>Glucose/GDH</td>
<td>EtOH 2.5</td>
<td>24</td>
<td>57</td>
<td>99:1</td>
<td>&gt;99</td>
</tr>
<tr>
<td>6</td>
<td>Glucose/GDH</td>
<td>1,4-dioxane 2.5</td>
<td>24</td>
<td>72</td>
<td>99:1</td>
<td>&gt;99</td>
</tr>
<tr>
<td>7</td>
<td>Glucose/GDH</td>
<td>1,4-dioxane 5</td>
<td>24</td>
<td>78</td>
<td>&gt;99</td>
<td>&gt;99</td>
</tr>
<tr>
<td>8</td>
<td>Glucose/GDH</td>
<td>1,4-dioxane 10</td>
<td>24</td>
<td>79</td>
<td>99:1</td>
<td>&gt;99</td>
</tr>
<tr>
<td>9</td>
<td>Glucose/GDH</td>
<td>1,4-dioxane 10</td>
<td>48</td>
<td>80</td>
<td>99:1</td>
<td>&gt;99</td>
</tr>
<tr>
<td>10</td>
<td>Glucose/GDH</td>
<td>MTBE 2.5</td>
<td>24</td>
<td>70</td>
<td>&gt;99</td>
<td>&gt;99</td>
</tr>
<tr>
<td>11</td>
<td>Glucose/GDH</td>
<td>MTBE 5</td>
<td>24</td>
<td>83</td>
<td>&gt;99</td>
<td>&gt;99</td>
</tr>
<tr>
<td>12</td>
<td>Glucose/GDH</td>
<td>MTBE 10</td>
<td>24</td>
<td>77</td>
<td>&gt;99</td>
<td>&gt;99</td>
</tr>
<tr>
<td>13</td>
<td>Glucose/GDH</td>
<td>MTBE 10</td>
<td>48</td>
<td>77</td>
<td>&gt;99</td>
<td>&gt;99</td>
</tr>
<tr>
<td>14</td>
<td>Glucose/GDH</td>
<td>THF 2.5</td>
<td>24</td>
<td>72</td>
<td>99:1</td>
<td>&gt;99</td>
</tr>
<tr>
<td>15</td>
<td>Glucose/GDH</td>
<td>THF 5</td>
<td>24</td>
<td>88</td>
<td>&gt;99</td>
<td>&gt;99</td>
</tr>
<tr>
<td>16</td>
<td>Glucose/GDH</td>
<td>THF 10</td>
<td>24</td>
<td>80</td>
<td>99:1</td>
<td>&gt;99</td>
</tr>
<tr>
<td>17</td>
<td>Glucose/GDH</td>
<td>THF 10</td>
<td>48</td>
<td>82</td>
<td>99:1</td>
<td>&gt;99</td>
</tr>
</tbody>
</table>

a For reaction details, see Materials and methods section; b Measured by High Performance Liquid Chromatography (HPLC); c Ratio of (1S,4S) and (1R,4R) to meso-diol; d Enantiomeric excess of (1S,4S)-1b.

Taking all these data into account, an exhaustive cosolvent screening was performed (entries 5–17). Ethanol (EtOH), 1,4-dioxane, methyl tert-butyl ether (MTBE), and tetrahydrofuran (THF) were chosen as suitable cosolvents for the biotransformation and employed in different quantities. When using 2.5% v/v of the organic solvent, for instance 1,4-dioxane, MTBE, and THF (entries 6, 10, and 14), better conversions than DMSO (entry 1) and EtOH (entry 5) were achieved, also affording excellent selectivities. An increase in the amount of these cosolvents from 2.5% to 5% v/v revealed even better conversions (entries 7, 11 and 15) and perfect diastereoselectivities (>99% de). Finally, the amount of cosolvent was increased up to 10% v/v (entries 8, 12, and 16), but unfortunately this did not lead to any improvement. Moreover, higher reaction times (from 24 h to 48 h, entries 9, 13, and 17) did not show better results. It must be pointed out that the hydroxy ketone intermediate was not observed in any case.

Commercial ADHs purchased from Codexis were also tested, as these enzymes have recently demonstrated interesting applications [48]. In this case, IPA was utilized as hydrogen source and phosphate buffer 125 mM pH 7.0 as suitable reaction medium. DMSO (2.5% v/v) was added again to solubilize the ketone. Thus, diketone 1a was a suitable substrate for five of them (69–89% conversion, 74–99% de, Table 2). ADH-P2-D03 led to the formation of the opposite enantiomer (1R,4R)-1b (entry 4) with excellent enantioselectivity and 74% de. Thus, by modifying the biocatalyst, both enantiomers of diol 1b could be achieved. Again, the hydroxy ketone intermediate was not detected.
Table 2. Bioreduction of 1,4-diphenylbutane-1,4-dione (1a) using commercially available alcohol dehydrogenases (ADHs)\textsuperscript{a}.

<table>
<thead>
<tr>
<th>Entry</th>
<th>ADH</th>
<th>Conversion (%)</th>
<th>\textit{de}\textsubscript{b,c}</th>
<th>ee (%)\textsubscript{b,d}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P1-B02</td>
<td>85</td>
<td>&gt;99</td>
<td>&gt;99</td>
</tr>
<tr>
<td>2</td>
<td>P1-B10</td>
<td>79</td>
<td>&gt;99</td>
<td>&gt;99</td>
</tr>
<tr>
<td>3</td>
<td>P1-B12</td>
<td>75</td>
<td>99:1</td>
<td>&gt;99</td>
</tr>
<tr>
<td>4</td>
<td>P2-D03</td>
<td>89</td>
<td>87:13</td>
<td>&gt;99\textsuperscript{c}</td>
</tr>
<tr>
<td>5</td>
<td>P2-D11</td>
<td>69</td>
<td>98:2</td>
<td>&gt;99</td>
</tr>
</tbody>
</table>

\(\textsuperscript{a}\) For reaction conditions, see Materials and methods section; \(\textsuperscript{b}\) Measured by HPLC; \(\textsuperscript{c}\) Ratio of (1\textit{S},4\textit{S}) and (1\textit{R},4\textit{R}) to meso-(1\textit{R},4\textit{S}); \(\textsuperscript{d}\) Enantiomeric excess of (1\textit{S},4\textit{S})-\textit{1b}; \(\textsuperscript{e}\) Enantiomeric excess of (1\textit{R},4\textit{R})-\textit{1b}.

Seeking further exploitation of the synthetic approach, first we considered the study of similar substrates that differed at the \textit{para} substitution of the aromatic ring. For this purpose, biotransformations with \textit{E. coli}/RasADH and substrates 2–5\textsubscript{a} were set up using the best conditions found in the reduction of diketone 1a (Table 1, entry 15, 5\% \textit{v}/\textit{v} THF, 30 °C, 24 h). The results are summarized in Scheme 2. In all cases, sole formation of (1\textit{S},4\textit{S})-diols 2–5\textsubscript{b} was observed (>99\% \textit{de} and >99\% \textit{ee}), with no detection of the corresponding hydroxy ketone intermediates in any case. For chlorinated substrate 4\textsubscript{a} (82\% conversion), the results were comparable to those obtained with the model substrate (88\% conversion). A small drop in the conversion was observed for methoxylated (2\textsubscript{a}) and methylated (3\textsubscript{a}) compounds (72\% and 77\%, respectively), while bulkier trifluoromethylated diketone 5\textsubscript{a} showed a significant loss of activity (50\% conversion).

Then, the study was also performed on substrates bearing \textit{meta} or \textit{ortho} substitution at the phenyl ring. Hence, diketones 6–8\textsubscript{a} were used as substrates for RasADH. However, very low yields (8\%) were observed for the corresponding enantiopure methoxylated derivatives (1\textit{S},4\textit{S})-6\textsubscript{b} and 8\textsubscript{b}. A special mention is deserved for \textit{ortho}-substituted diketone 8\textsubscript{a}, that provided hydroxy ketone 8\textsubscript{c} at higher extent (36\%) than the corresponding diol 8\textsubscript{b}. Regarding the \textit{m}-chloro derivative 7\textsubscript{a}, that showed higher enzymatic conversion (22\%), two different experiments were also carried out. On the one hand, the amount of enzyme was doubled leading to the formation of (1\textit{S},4\textit{S})-7\textsubscript{b} in 34\% conversion after 24 h. On the other hand, the temperature was raised up to 40 °C, obtaining (1\textit{S},4\textit{S})-7\textsubscript{b} in 43\% conversion after 24 h. These results showed that the substitution pattern had an effect on the enzymatic activity, achieving the most valuable results for the \textit{para}-substituted diketones.

Next, the bioreductions of diketones 2–5\textsubscript{a} were also attempted with the commercially available ADHs reported in Table 2. Unfortunately, no conversion was observed in any case, demonstrating the difficulty of these bioconversions, only achieved with RasADH.

Finally, preparative biotransformations up to 100-mg scale were carried out using \textit{E. coli}/RasADH under the optimized reaction conditions (Figure 1), using THF (5\% \textit{v}/\textit{v}) as cosolvent at 30 °C and glucose/GDH as NADPH regeneration system. After 24 h, the conversions obtained were between 40\% and 90\%, isolating the corresponding diastereo- and enantiopure diols (1\textit{S},4\textit{S})-1\textsubscript{b} in moderate to high yields. The highest yield was obtained for the unsubstituted diol (1\textit{S},4\textit{S})-1\textsubscript{b}, which was recovered in 73\% yield, while the \textit{m}-chloro compound (7\textsubscript{b}) led to the lowest yield (10\%).
Scheme 2.Selective bioreduction of diketones 2–8a using E. coli/RasADH and tetrahydrofuran (THF) as cosolvent.

Figure 1. Conversions and isolated yields for the RasADH-catalyzed preparative biotransformations to obtain the optically active diols (1S,4S)-1–5,7b.
3. Conclusions

A series of 1,4-diaryl-1,4-diols were synthesized via ADH-catalyzed bioreduction from the corresponding bulky 1,4-diaryl-1,4-diketones. Among the different enzymatic preparations used, ADH from Ralstonia sp. (RasADH) overexpressed in E. coli afforded the best results in terms of conversions and diastereo- and enantiomeric excess, usually obtaining the (1S,4S)-diols, and only observing the formation of the hydroxy ketone intermediate in the case of the o-methoxylated derivative. The cofactor regeneration system and the addition of an organic cosolvent were studied, showing that glucose/GDH and THF were the best options. Herein we have shown that the bioreduction can be a useful alternative under mild conditions to obtain these appealing chiral compounds, which can be further employed as synthons for the preparation of other valuable derivatives.

4. Materials and Methods

As for the substrates and products studied in this contribution, HPLC separations, HPLC chromatograms of optically active 1,4-diaryl-1,4-diols and NMR spectra, please see the Supplementary Materials.

4.1. General Materials and Methods

The 4′-Substituted acetophenones and α-bromo-4′-substituted acetophenones were purchased from TCI Europe (Zwijndrecht, Belgium). NADPH as enzyme cofactor and all the other chemical reagents were obtained with the highest quality available from Sigma-Aldrich-Fluka (Steinheim, Germany). Alcohol dehydrogenases and GDH were obtained from Codexis Inc., (Redwood City, CA, USA). The production of E. coli overexpressed ADHs has been previously reported and these enzymatic preparations have been provided by Prof. Wolfgang Kroutil (University of Graz) [35,41,43].

NMR spectra were recorded on a Bruker AV300 MHz spectrometer (Bruker Co., Faellanden, Switzerland). All chemical shifts (δ) are given in parts per million (ppm) and referenced to the residual solvent signal as internal standard. Measurement of the optical rotation values was carried out at 590 nm on a Autopol IV Automatic polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA). High performance liquid chromatography (HPLC) analyses were performed for conversion, enantiomeric excess, and diastereomeric excess value measurements using a Hewlett Packard 1100 chromatograph UV detector at 210 nm (Agilent Technologies, Inc., Wilmington, DE, USA). As chiral columns, Chiralpak AD-H (25 cm × 4.6 mM) and Chiralpak IA (25 cm × 4.6 mM) were used (Chiral Technologies, Mainz, Germany). Thin-layer chromatography (TLC) analyses were conducted with Merck Silica Gel 60 F254 precoated plates (Merck KGaA, Darmstadt, Germany) and visualized with UV and potassium permanganate stain. Column chromatography purifications were performed using Merck Silica Gel 60 (230–400 mesh, Merck KGaA, Darmstadt, Germany).

4.2. General Procedure for the Synthesis of 1,4-Diaryl-1,4-Diketones 1–8a

For the preparation of diketones 1–8a we followed a similar methodology to the one described in the literature [40]. Commercial anhydrous ZnCl2 (2.72 g, 20 mMol) was placed into a one-neck 250-mL round-bottom flask and dried by melting under vacuum (1 torr) at 250–350 °C for 15–20 min. After cooling under vacuum to room temperature, toluene (10 mL), diethylamine (1.03 mL, 10 mmol) and tBuOH (0.95 mL, 10 mmol) were successively added. The mixture was stirred until zinc chloride was fully dissolved (approx. 2 h), and then the corresponding acetophenone (8.0 mMol) and α-bromoacetophenone (5.0 mMol) were successively added. The mixture was stirred at room temperature for 4 days. After this, CH2Cl2 (300 mL) was added and the resulting organic phase was successively washed with an aqueous H2SO4 2.0 M solution (2 × 120 mL), water (1 × 150 mL) and brine (1 × 150 mL). The organic phase was dried over Na2SO4, filtered, and the solvents were evaporated under reduced pressure. Crude solids, except compounds 6a and 8a that were purified by column chromatography (Hexane/EtOAc 4:1), were purified by crystallization in absolute EtOH in
order to yield the corresponding 1,4-diaryl-1,4-diketones (see Supplementary Materials) 1a (0.69 g, 58% yield), 2a (0.36 g, 24%), 3a (0.33 g, 25%), 4a (0.51 g, 33%), 5a (0.91 g, 49%), 6a (0.19 g, 13%), 7a (0.79 g, 52%) and 8a (0.11 g, 8%) [38-40,49-51].

4.3. General Procedure for the Synthesis of Racemic 1,4-Diaryl-1,4-Diols 1–8b

Diketones 1–8a (0.3 mMol) were dissolved in THF (2.0 mL) and NaBH₄ (90.8 mg, 2.4 mMol, 8.0 equiv.) was slowly added at room temperature. Reactions were stirred for 2–4 h until completion. After this, an aqueous HCl 1.0 M solution (2.0 mL) was added and the mixtures were extracted with CH₂Cl₂ (2 × 5 mL). The organic phases were dried over Na₂SO₄, filtered and the solvents were evaporated under reduced pressure. The reaction crude was purified by column chromatography (EtOAc/hexane mixtures), isolating the racemic 1,4-diaryl-1,4-diols (±)-1b (61.7 mg, 85% yield), (±)-2b (65.2 mg, 72%), (±)-3b (65.6 mg, 81%), (±)-4b (71.9 mg, 77%), (±)-5b (102.1 mg, 90%), (±)-6b (30.0 mg, 52%), (±)-7b (58.3 mg, 62%) and (±)-8b (33.0 mg, 66%).

4.4. General Procedure for the Synthesis of Racemic 4-Hydroxy-1,4-Bis(2-methoxyphenyl)butan-1-one 8c

Diketone 8a (0.13 mMol) was dissolved in THF (0.9 mL) and NaBH₄ (21 mg, 0.54 mMol, 4.0 equiv.) was added at room temperature. The reaction was stirred for 30 min until the formation of the hydroxy ketone was observed by TLC analysis. After this, an aqueous HCl 1.0 M solution (2.0 mL) was added and the mixture was extracted with CH₂Cl₂ (2 × 5 mL). The organic layers were dried over Na₂SO₄, filtered and the solvents were evaporated under reduced pressure. The reaction crude was purified by column chromatography (EtOAc/hexane mixtures), isolating the racemic hydroxy ketone (±)-8c (2 mg, 5% yield).

4.5. General Procedure for the Enzymatic Conversion of 1,4-Diaryl-1,4-Diols 1–8b Using Overexpressed E. coli/RasADH

Lyophilized E. coli/RasADH cells (15–30 mg), the cosolvent (2.5%–10% v/v), 1 mM NADP⁺ (60 µL of a 10 mM stock solution), 50 mM glucose (60 µL of a 500 mM stock solution) and glucose dehydrogenase (5 U) were added into an Eppendorf tube containing 1,4-diaryl-1,4-diketones (1–8a, 25 mM) in Tris-HCl buffer 50 mM pH 7.5 (420 µL). The reaction was shaken at 30–40 °C and 250 rpm for 24–48 h. After this time, the mixture was extracted with ethyl acetate (2 × 500 µL), the organic layers separated by centrifugation (2 min, 5700 × g), combined and finally dried over Na₂SO₄. Conversion, diastereomeric excess and enantiomeric excess values of 1,4-diaryl-1,4-diols 1–8b were determined by HPLC (see Supplementary Materials).

4.6. General Procedure for the Bioreduction of 1,4-Diphenylbutane-1,4-Dione 1a Using Commercial Alcohol Dehydrogenases

In a 2.0 mL Eppendorf tube, KRED (2 mg) was added to 900 µL phosphate buffer 125 mM pH 7.0 (1.25 mM mgSO₄, 1 mM NADP⁺) containing 1,4-diphenylbutane-1,4-dione (1a, 25 mM), DMSO (25 µL) and 1PrOH (100 µL). The reaction was incubated at 250 rpm and 30 °C for 24 h. Then, the mixture was extracted with ethyl acetate (2 × 500 µL), the organic layers separated by centrifugation (2 min, 5700 × g), combined and finally dried over Na₂SO₄. Conversion, diastereomeric excess and enantiomeric excess values of 1,4-diphenylbutane-1,4-diol (1b) were determined by HPLC.

4.7. Preparative Bioreductions of 1,4-Diarylbutane-1,4-Diones 1–5a, 7a, and 8a Using Overexpressed E. coli/RasADH

Lyophilized E. coli/RasADH cells (50 mg for 1,4-diketone 1a, 20 mg for 1,4-diketones 2–4a, 100 mg for 1,4-diketone 5a, and 150 mg for 1,4-diketone 7a). THF (5% v/v), NADP⁺ (1 mM), glucose (50 mM), and GDH (50–100 U), were added into an Erlenmeyer flask containing a suspension of the corresponding 1,4-diketone (25 mM) in Tris-HCl buffer 50 mM pH 7.5. The reaction was incubated at 30 °C and 250 rpm for 24 h (1a–5a) or at 40 °C and 250 rpm for 48 h (7a). Then, the mixture was
extracted with ethyl acetate (3 × 15 mL). The organic layers were separated by centrifugation (5 min, 4000×g), combined, and finally dried over Na2SO4. The reaction crude was purified by column chromatography (EtOAc/hexane mixtures), isolating the enantiopure (1S,4S)-diols in moderate to high yields (10–73%).

(−)-(1S4S)-1,4-Diphenylbutane-1,4-diol (1S4S)-1b. Yield: 37 mg (73%). Rf = 0.36 (40% EtOAc/hexane). Mp: 74–75 °C. 1H NMR (300.13 MHz, CDCl3): δ 1.76–1.96 (m, 2H), 1.88–2.02 (m, 2H), 2.59 (s, 2OH), 4.72 (dd, JHH = 6.5, 3.9 Hz, 2H), 7.08–7.50 (m, 10H) ppm. 13C NMR (300.13 MHz, CDCl3): δ 35.9 (2CH2), 74.6 (2CH2), 125.8 (4CH), 127.5 (2CH), 128.4 (4CH), 144.6 (2C) ppm. HRMS (ESI+, m/z): calcd for (C16H18NaO2)+ (M + Na)+ 265.1204; found 265.1199. [α]D21 = −57.2 (c = 0.5, CHCl3), described in the literature [32]; [α]D25 = −59.0 (c = 1.0, CHCl3) for syn-(S,S)-diol.

(−)-(1S4S)-1,4-Bis(4-methoxyphenyl)butane-1,4-diol (1S4S)-2b. Yield: 9 mg (41%). Rf = 0.21 (40% EtOAc/hexane). Mp: 114–115 °C. 1H NMR (300.13 MHz, CDCl3): δ 1.71–1.85 (m, 2H), 1.85–2.00 (m, 2H), 3.82 (s, 6H), 4.68 (m, 2H), 6.88 (d, JHH = 8.5, 4H), 7.27 (d, JHH = 8.1 Hz, 4H) ppm. 13C NMR (300.13 MHz, CDCl3): δ 34.4 (2CH2), 53.6 (2CH2), 81.0 (2CH), 121.3 (2CH), 135.2 (2C), 159.1 (2C) ppm. HRMS (ESI+, m/z): calcd for (C18H22NaO4)+ (M + Na)+ 325.1415; found 325.1410. [α]D21 = −43.0 (c = 0.1, CHCl3), described in the literature [11]; [α]D25 = +41.6 (c = 1.0, CHCl3) for syn-(R,R)-diol.

(−)-(1S4S)-1,4-Bis(4-chlorophenyl)butane-1,4-diol (1S4S)-3b. Yield: 14 mg (69%). Rf = 0.27 (40% EtOAc/hexane). Mp: 114–115 °C. 1H NMR (300.13 MHz, CDCl3): δ 1.72–1.80 (m, 2H), 1.88–2.04 (m, 2H), 2.28 (s, 2OH), 2.36 (s, 6H), 4.70 (dd, JHH = 6.8, 4.4 Hz, 2H), 7.16 (d, JHH = 8.0 Hz, 4H), 7.24 (d, JHH = 8.0 Hz, 4H) ppm. 13C NMR (300.13 MHz, CDCl3): δ 21.1 (2CH), 35.8 (2CH2), 74.5 (2CH), 125.8 (4CH), 129.1 (4CH), 137.2 (2C), 141.7 (2C) ppm. HRMS (ESI+, m/z): calcd for (C18H22NaO2)+ (M + Na)+ 293.1517; found 293.1512. [α]D21 = −45.2 (c = 0.2, CH2Cl2), described in the literature [4]; [α]D25 = −47.0 (c = 1.0, CH2Cl2) for syn-(S,S)-diol.

(−)-(1S4S)-1,4-Bis(4-chlorophenyl)butane-1,4-diol (1S4S)-4b. Yield: 11 mg (57%). Rf = 0.45 (40% EtOAc/hexane). Mp: 112–113 °C. 1H NMR (300.13 MHz, CDCl3): δ 1.69–2.01 (m, 4H), 2.31 (s, 2OH), 4.71 (d, JHH = 5.9 Hz, 2H), 7.09–7.46 (m, 8H) ppm. 13C NMR (300.13 MHz, CDCl3): δ 35.8 (2CH2), 73.8 (2CH), 127.1 (4CH), 128.5 (4CH), 133.2 (2C), 142.9 (2C) ppm. HRMS (ESI+, m/z): calcd for (C18H16Cl2NaO2)+ (M + Na)+ 333.0425; found 333.0419. [α]D21 = −22.1 (c = 0.2, CHCl3), described in the literature [11]; [α]D25 = +24.4 (c = 1.05, CHCl3) for syn-(R,R)-diol.

(−)-(1S4S)-1,4-Bis(4-trifluoromethylphenyl)butane-1,4-diol (1S4S)-5b. Yield: 63 mg (64%). Rf = 0.51 (40% EtOAc/hexane). Mp: 159–160 °C. 1H NMR (300.13 MHz, MeOD): δ 1.67–1.77 (m, 2H), 1.78–1.92 (m, 2H), 3.30 (s, 2OH), 4.73 (apparent t, JHH = 5.6 Hz, 2H), 7.49 (d, JHH = 8.2 Hz, 4H), 7.60 (d, JHH = 8.2 Hz, 4H) ppm. 13C NMR (300.13 MHz, MeOD): δ 34.8 (2CH2), 72.5 (2CH), 124.4 (q, JCF = 271.2 Hz, 2CF3), 124.7 (q, JCF = 3.6 Hz, 4CH), 126.1 (4CH), 128.9 (q, JCF = 32.0 Hz, 2C), 149.6 (2C) ppm. HRMS (ESI+, m/z): calcd for (C18H16F6Na2O2)+ (M + Na)+ 401.0952; found 401.0951. [α]D21 = −20.1 (c = 0.4, CHCl3), described in the literature [3]; [α]D25 = +19.0 (c = 0.1, CHCl3) for syn-(R,R)-diol.

(−)-(1S4S)-1,4-Bis(3-chlorophenyl)butane-1,4-diol (1S4S)-7b. Yield: 8 mg (10%). Rf = 0.35 (40% EtOAc/hexane). 1H NMR (300.13 MHz, CDCl3): δ 1.80–1.93 (m, 4H), 2.54 (s, 2OH), 4.71 (m, 2H), 7.02–7.51 (m, 8H) ppm. 13C NMR (300.13 MHz, CDCl3): δ 35.8 (2CH2), 74.0 (2CH), 124.0 (2CH), 126.1 (2CH), 127.8 (2CH), 129.9 (2CH), 134.5 (2C), 146.7 (2C) ppm. HRMS (ESI+, m/z): calcd for (C16H16Cl2NaO2)+ (M + Na)+ 333.0425; found 333.0426. [α]D21 = −41.0 (c = 0.1, CHCl3).

To obtain the hydroxy ketone 8c, lyophilized E. coli/RasADH cells (80 mg), THF (5% v/v), NADP+ (1 mM), glucose (50 mM), and GDH (50 U), were added into an Erlenmeyer flask containing a suspension of the 1,4-diketone 8a (20 mg, 25 mM) in Tris-HCl buffer 50 mM pH 7.5. The reaction was
incubated at 30 °C and 250 rpm for 24 h. Then, the mixture was extracted with ethyl acetate (3 × 15 mL) and the organic layers were separated by centrifugation (5 min, 4000 × g), combined and finally dried over Na₂SO₄. The reaction crude was purified by column chromatography (EtOAc/hexane mixtures), isolating 8c (2 mg, 10% yield).

4-Hydroxy-1,4-bis(2-methoxyphenyl)butan-1-one (8c). Rf = 0.41 (40% EtOAc/hexane).

1H NMR (300.13 MHz, CDCl₃): δ 2.13–2.36 (m, 2H), 3.00 (s, OH), 3.05–3.29 (m, 2H), 3.85 (s, OCH₃), 3.88 (s, OCH₃), 6.79–7.07 (m, 4H), 7.25 (m, 1H), 7.38 (d, JHH = 7.5 Hz, 1H), 7.40–7.51 (t, JHH = 7.8 Hz, 1H), 7.69 (dd, JHH = 7.7, 1.8 Hz, 1H) ppm. HRMS (ESI+, m/z): calcld for (C₁₈H₂₀NaO₄)⁺ (M + Na)⁺ 323.1259; found 323.1260.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4344/8/4/150/s1. Substrates and products studied in this contribution, 2. HPLC separations, 3. HPLC chromatograms of optically active 1,4-diaryl-1,4-diols, 4. NMR spectra.

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Conflicts of Interest: The authors declare no conflict of interest.

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