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Enzymatic synthesis of all stereoisomers of 1-phenylpropane-1,2-diol

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Abstract—A stereoselective two-step enzymatic synthesis of all four stereoisomers of 1-phenylpropane-1,2-diol starting from benzaldehyde and acetaldehyde is described. By using one of four possible combinations of a lyase followed by an alcohol dehydrogenase, each diol is accessible separately. © 2002 Published by Elsevier Science Ltd.

1. Introduction

Optically active vicinal diols are interesting building blocks in asymmetric synthesis. Chemical routes towards *vic*-diols in terms of stereospecificity and enantioselectivity include the catalytic *cis*-dihydroxylation of olefins with OsO_4[1,2] and the catalytic asymmetric transfer hydrogenation of diketones.[3] Amongst the biocatalytic methods for the synthesis of *vic*-diols are the hydrolysis of epoxides with epoxide hydrolases,[4,5] the kinetic resolution of racemic *vic*-diols achieved by enzymatic oxidation[6] and the enzymatic and microbial reduction of 1,2-diketones and α-hydroxy ketones.[7,8] A drawback of the above mentioned methods, is that only one or two of the diol stereoisomers are accessible in good diastereomeric purity from a given starting material. We have now synthesised all four possible 1-phenylpropane-1,2-diol stereoisomers separately by employing a combination of enantioselective lyases and diastereoselective alcohol dehydrogenases in succession. Moreover, simple molecules like benzaldehyde and acetaldehyde are used as starting materials.

2. Results and discussion

The thiamine diphosphate (ThDP)-dependent enzymes benzaldehyde lyase[9,10] (BAL, EC 4.1.2.38) and benzoylformate decarboxylase[11] (BFD, EC 4.1.1.7) are both able to catalyse enantioselective C–C bond formations between acetaldehyde and benzaldehyde, leading to 2-hydroxy-ketones. The BFD-catalysed reaction yields (2*S*)-hydroxy-1-phenyl-propanone (*S*)-**1** with an e.e. of 94% (above 99% e.e. after recrystallisation) whereas the BAL-catalysed reaction supplies the (2*R*)-hydroxy-1-phenyl-propanone (*R*)-**1** with an e.e. >99%. In the latter case, the formation of an intermediate benzoin is proposed.

Upon screening secondary alcohol dehydrogenases that would accept both enantiomers of **1** and reduce them diastereoselectively to the corresponding 1-phenylpropane-1,2-diols, we found the recombinant *Lactobacillus brevis* alcohol dehydrogenase (rec*Lb*-ADH, EC 1.1.1.2) was able to catalyse the reduction of the prochiral keto function of both (*S*)-**1** and (*R*)-**1**, generating an (*S*)-configured stereogenic centre in the products (1*S*,2*S*)-phenylpropane-1,2-diol (1*S*,2*S*)-**2**, and (1*S*,2*R*)-phenylpropane-1,2-diol (1*S*,2*R*)-**3**, respectively. Alcohol dehydrogenase from the thermophilic *Thermoanaerobium species* (*Th. sp.*-ADH, EC 1.1.1.2) was found to reduce the keto function of both (*S*)-**1** and (*R*)-**1** furnishing isomeric products with (*R*)-configuration at the newly formed stereogenic centre of (1*R*,2*S*)-phenylpropane-1,2-diol (1*R*,2*S*)-**3** and (1*R*,2*R*)-phenylpropane-1,2-diol (1*R*,2*R*)-**2**, respectively. In this manner it is possible to synthesise all four stereoisomers of 1-phenylpropane-1,2-diol with high enantiomeric purity and good yields (see Scheme 1).

A similar approach using pyruvate decarboxylase (PDC) as an alternative catalyst for the C–C bond formation is conceivable and has been demonstrated for yeast cells containing PDC and alcohol dehydrogenases albeit with only the (1*R*,2*S*)-**3**-diol as the major isolated product [(1*R*,2*S*)-**3**:(1*R*,2*R*)-**2**:(1*S*,2*S*)-

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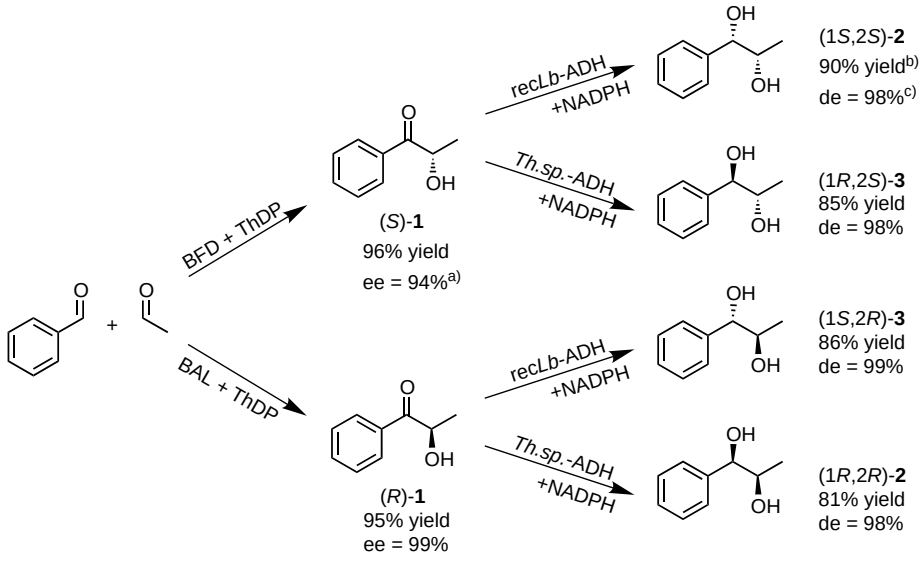

Scheme 1. Enantio- and diastereoselective enzymatic synthesis of 1-phenylpropane-1,2-diol stereoisomers in a reaction cascade. (a) After crystallisation e.e. >99%; (b) Yields of diols are given as overall yields for the two steps; (c) D.e.s of diols are those for the crude reaction.

2:(1*S*,2*R*)-**3** = 89:6:5:0].[7] A problem that arises in a stepwise approach using purified PDC is the susceptibility of the intermediate (1*R*)-hydroxy-1-phenylpropanone for oxidation to the dione during work-up. Furthermore, to the best of our knowledge, the (*S*)-enantiomer is not accessible in high enantiomeric excess by this method so far,[12] making this approach unfavourable for the diol synthesis.

Both alcohol dehydrogenases used for hydroxy ketone reduction are NADP(H)-dependent. In anticipation of possible scale-up we decided to demonstrate the feasibility of an integrated cofactor regeneration system in an enzyme-coupled (double-enzyme system) and substrate-coupled (one-enzyme system) approach. When using *Th. sp.*-ADH for reduction at 40°C, regeneration of the cofactor was achieved with an excess (100-fold higher concentration) of 2-propanol in the substrate-coupled approach. The relatively high reaction temperatures of above 40°C required for sufficient activity when employing *Th. sp.*-ADH limits the alternatives for cofactor regeneration to the enzyme itself or other thermo-stable NADP(H) dependent enzymes. It must be pointed out however, that at these relatively high temperatures, the stability of the cofactor NADPH as well as the enzyme are limited.

In the case of reduction with rec*Lb*-ADH at 20°C it could be shown that both an enzyme- and substrate-coupled regeneration system are applicable. The advantage of the NADP(H) dependent FDH (EC 1.2.1.2)[13,14] system lies in the favourable equilibrium strongly shifted towards CO_2 and NADPH.[15] In addition the CO_2 by-product can be easily removed and, compared to the 2-propanol–acetone system, does not inhibit the regeneration or reduction reaction as acetone does. Table 1 summarises the kinetic data for reduction and preferred regeneration system.

Table 1. Kinetic data for the reduction and regeneration reactions

Product	Kinetic parameters of enzyme–substrate pair under reaction conditions	System used for NADPH regeneration and selected kinetic parameters under reaction conditions
(1*S*,2*S*)-**2**	Reduction of (*S*)-**1** with rec*Lb*-ADH $V_{max} = 1.50 \pm 0.05$ U/mg $K_{M,\ (S)\text{-}1} = 2.36 \pm 0.30$ mM $K_{M,\ NADPH} = 0.41 \pm 0.02$ mM	FDH in the presence of 300 mM HCOONa $V_{max} = 1.01 \pm 0.08$ U/mg $K_{M,\ HCOO^-} = 22.6 \pm 3.1$ mM $K_{M,\ NADP^+} = 0.23 \pm 0.03$ mM
(1*R*,2*S*)-**3**	Reduction of (*R*)-**1** with rec*Lb*-ADH $V_{max} = 1.09 \pm 0.05$ U/mg $K_{M,\ (R)\text{-}1} = 7.48 \pm 1.10$ mM	
(1*S*,2*R*)-**3**	Reduction of (*S*)-**1** with *Th. sp.*-ADH $V_{max} = 1.35 \pm 0.02$ U/mg $K_{M,\ (S)\text{-}1} = 0.1 \pm 0.02$ mM	*Th. sp.*-ADH in the presence of 100 mM 2-propanol $V_{max} = 8.10 \pm 0.18$ U/mg $K_{M,\ 2\text{-prop.}} = 3.4 \pm 0.4$ mM
(1*R*,2*R*)-**2**	Reduction of (*R*)-**1** with *Th. sp.*-ADH $V_{max} = 0.90 \pm 0.05$ U/mg $K_{M,\ (R)\text{-}1} = 0.21 \pm 0.04$ mM	

Fortunately, the specific activities of both ADHs towards the reduction of (*S*)-**1** and (*R*)-**1** lie in the same order of magnitude. In consequence, we did not find a significant preferential reduction of one enantiomer of **1** by either ADH. Thus, all stereoisomers of the diol are equally well accessible. Moreover, a racemic resolution of **1** by selective reduction with the ADHs mentioned should not be possible.

A continuous reactor set-up for successive C–C bond formation and reduction reactions is the object of our current investigations and the economical use of enzyme activity at high total turnover numbers for NADPH as well as high space-time yields are the main objectives for the reactor design.

3. Experimental

Purification of the enzymes BFD and BAL as well as the synthesis of (*R*)-**1** was carried out as indicated previously.[10,11] In the case of rec*Lb*-ADH the crude extract was used,[16] which is commercially available from Jülich Fine Chemicals, Germany. *Th. sp.*-ADH and NADP-dependent FDH were purchased from Jülich Fine Chemicals, Germany. Separation of the underivatised diols was carried out by gas chromatography on a 25 m×0.25 mm CP-Chirasil-DEX CB column (Varian, Germany); oven temp. = 130°C, injection temp = 250°C, $\tau_{(1S,2S)\text{-}\mathbf{2}} = 20.0$ min, $\tau_{(1R,2R)\text{-}\mathbf{2}} = 21.9$ min, $\tau_{(1S,2R)\text{-}\mathbf{3}} = 22.9$ min, $\tau_{(1R,2S)\text{-}\mathbf{3}} = 23.9$ min. For the determination of % conversion from the reaction a 25×0.4 cm RP-8 column (Merck, Darmstadt, Germany) was used: 20% acetonitrile in aq. 0.2% TEA-buffer pH 3, 1.1 mL/min, $\tau_{((1R,2S)\text{-}\mathbf{3} \text{ and } (1S,2R)\text{-}\mathbf{3})} = 7.9$ min, $\tau_{((1S,2S)\text{-}\mathbf{2} \text{ and } (1R,2R)\text{-}\mathbf{2})} = 8.9$ min.

3.1. Synthesis of (*S*)-1

Benzaldehyde (18.67 g, 175 mmol) and acetaldehyde (66.0 g, 1500 mmol) were dissolved in phosphate buffer (5 L, 50 mM, pH 8) containing ThDP (5.0 g, 11 mmol) and $MgSO_4 \cdot 7\ H_2O$ (0.6 g, 2.4 mmol). The reaction was started by addition of BFD (114 U) and allowed to stand for 48 h at RT for complete conversion. To prevent foaming during work-up, the dissolved enzyme was filtered off with a YM 10 membrane in an Amicon cell (Millipore, Eschborn, Germany). The filtrate was washed with EtOAc and the combined organic layers were dried over $MgSO_4$ and evaporated under reduced pressure to give a crude oil, which was purified by recrystallisation from *i*-hexane; yield = 25.2 g (96%), e.e. >99%. $[\alpha]_D^{20} = -86$ (*c* 2, $CHCl_3$), [lit.[11] $[\alpha]_D^{25} = -79.2$ (*c* 0.016, $CHCl_3$) for e.e. = 95%].

3.2. Synthesis of (1*S*,2*S*)-2 and (1*S*,2*R*)-3; representative example for the enzymatic reduction with rec*Lb*-ADH and FDH coupled NADPH regeneration

Compound (*S*)-**1** (983 mg, 6.5 mmol) was dissolved in phosphate buffer (250 mL, 100 mM, pH 7) containing sodium formate (4.08 g, 60.1 mmol), $NADP^+$ (0.5 mg, 0.6 mmol) and 1,4-dithiothreitol (DTT, 38 mg, 0.2 mmol). FDH (6.2 U, 0.025 U/mL; 1 U of FDH reduces 1 μmol of $NADP^+$ to NADPH in the presence of sodium formate (300 mM) and $NADP^+$ (1 mM) at 30°C, pH 7) was added to the stirred buffer at 20°C followed by the addition of 10 U (0.04 U/mL) of rec*Lb*-ADH after approx. 30 min. A conversion of over 94% was achieved after 24 h. For the synthesis of (1*S*,2*R*)-**3**, compound (*R*)-**1** (200 mg, 1.3 mmol) was used, maintaining the same concentrations for all reactants as in the synthesis of (1*S*,2*S*)-**2**. The diastereomeric excess of the diols determined from the reaction medium were 98 and 99% for (1*S*,2*S*)-**2** and (1*S*,2*R*)-**3**, respectively. For facile work-up the dissolved enzyme was filtered off with a YM 10 membrane in an Amicon cell. The filtrate was saturated with NaCl and washed with Et_2O (4×50 mL). The combined organic layers were dried over $MgSO_4$ and evaporated under reduced pressure to give a crude oil. The crude oil was purified by column chromatography using a gradient of 10–80% EtOAc in *i*-hexane.

(1*S*,2*S*)-**2**; yield = 936 mg (94%), d.e. = 98%, ^{1}H NMR (300 MHz), $CDCl_3$: δ 7.20 (5H, m), 4.15 (1H, d, $J = 7.7$ Hz), 3.82 (2H, br), 3.67 (1H, dq, $J = 6.4$ Hz), 0.85 (3H, d, $J = 6.3$ Hz), $[\alpha]_D^{20} = +54.3$ (*c* 1.9, $CHCl_3$), [lit.[8] $[\alpha]_D^{20} = +55.9$ (*c* 1.9, $CHCl_3$)].

(1*S*,2*R*)-**3**; yield = 184 mg (91%), d.e. = 99%, ^{1}H NMR (300 MHz), $CDCl_3$: δ 7.3 (5H, m), 4.6 (1H, d, $J = 4.0$ Hz), 3.9 (1H, m), 3.10 (2H, br), 1.0 (3H, d, $J = 6.4$ Hz), $[\alpha]_D^{20} = +36.1$ (*c* 2.52, $CHCl_3$), [lit.[17] $[\alpha]_D^{22} = +33$ (*c* 3.2, $CHCl_3$)].

3.3. Synthesis of (1*R*,2*S*)-3 and (1*R*,2*R*)-2

3.3.1. Representative example for the reduction reaction using *Th. sp.*-ADH and substrate coupled NADPH regeneration. (*S*)-**1** or (*R*)-**1** (150 mg, 1 mmol) were dissolved in phosphate buffer (100 mL, 100 mM, pH 8) containing 766 μL 2-propanol (10 mmol), 0.08 mg $NADP^+$ (0.1 mmol) and DTT (15 mg, 0.08 mmol). The reaction was started by addition of *Th. sp.*-ADH (7.5 U, 0.075 U/mL; 1 U of *Th. sp.* reduces 1 μmol (*S*)-**1** or (*R*)-**1** to diol in one minute at 40°C, pH 8). A conversion of over 90% was achieved after 24 h. To enable facile work-up the dissolved enzyme was filtered off with a YM 10 membrane in an Amicon cell. The filtrate was saturated with NaCl and washed with Et_2O (4×50 mL). The combined organic layers were dried over $MgSO_4$ and evaporated under reduced pressure to give a crude oil. The crude oil was purified by column chromatography using a gradient of 10–80% EtOAc in *i*-hexane.

(1*R*,2*S*)-**3**; yield = 135 mg (89%), d.e. = 98%, ^{1}H NMR (300 MHz), $CDCl_3$: δ 7.33 (5H, m), 4.65 (1H, d, $J = 4.3$ Hz), 4.0 (1H, m), 2.60–2.0 (2H, br), 1.05 (3H, d, $J = 6.4$ Hz), $[\alpha]_D^{20} = -29.9$ (*c* 2.4, $CHCl_3$), [lit.[18] $[\alpha]_D^{20} = -35$ (*c* 1, $CHCl_3$)].

(1*R*,2*R*)-**2**; yield = 129 mg (85%), d.e. = 98%, ^{1}H NMR (300 MHz), $CDCl_3$: δ 7.30 (5H, m), 4.32 (1H, d, $J = 7.5$ Hz), 3.82 (1H, dt), 3.13 (2H, br), 1.0 (3H, d, $J = 6.4$

Hz), $[\alpha]_D^{20} = -51.3$ (c 3.5, $CHCl_3$), [lit.[6] $[\alpha]_D = -55.9$ (c 1.9, $CHCl_3$)]

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