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Tetrahedron: *Asymmetry* 15 (2004) 1685–1692

Enzyme directed diastereoselectivity in chemical reductions: studies towards the preparation of all four isomers of 1-phenyl-1,3-butanediol

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Received 9 February 2004; accepted 13 April 2004

Abstract—Enzymes play an important role in guiding the diastereoselectivity of the final products during the chemical reduction of the intermediates (*R*)- and (*S*)-3-hydroxy-1-phenyl-1-butanone, prepared by bioreduction of 1-phenyl-1,3-butadione. For example, the presence of an oxidoreductase such as *Pichia capsulata* during a one pot, two step, enzymo-chemical reduction of 1-phenyl-1,3-butadione produced corresponding (1*R*,3*S*)-1-phenyl-1,3-butanediol (de ~ 98%), similarly *Zygosaccharomyces rouxii* furnished (1*S*,3*R*)-1-phenyl-1,3-butanediol (de ~ 98%). On the other hand chemical reduction of (*R*)- and (*S*)-3-hydroxy-1-phenyl-1-butanone after the exclusion of the enzymes resulted in complete loss of diastereoselectivity, leading to the formation of all four isomers of 1-phenyl-1,3-butanediol. Moreover the yields of the final products are higher in the one-pot transformations than in the two step sequential reactions.

1. Introduction

Stereoselective transformations using biocatalysts[1a] are now regularly exploited in the preparation of chiral synthons in natural products synthesis. Use of inert solid supports such as silica gel and alumina to improve reactivity and yield in the reduction of keto functions to the corresponding secondary alcohols by chemical as well as enzymatic methods has been a much studied reaction.[1b] The presence of a protein as a guide to regioselective reduction is a comparatively recent development. Chemoreduction in the presence of BSA (*Bovine serum albumin*) for improving regioselectivity particularly of natural products such as sterols has been reported.[1c] On the other hand the influence of crude enzymes on the diastereoselectivity profile of chemical reactions such as reductions has not been explored. Herein we describe results obtained during one-pot two step enzymo-chemical reduction reactions of the important substrate 1-phenyl-1,3-butadione **1**. The results of these transformations unambiguously demonstrate that crude enzymes play an important role in the stereoselectivity during the formation of diastereoisomers of 1-phenyl-1,3-butanediol. Both acyclic and cyclic 1,3-diols are of much interest to synthetic chemists since these molecules form the basic skeleton of natural products such as polyene and polyol macrolide antibiotics, for example, Compactin, Rifamycin[2a] and Lonomycin A, etc.[2b] They are also the precursors in the synthesis of some important natural products such as pheromones. Thus protection of diols followed by conversion to chiral hydroxyketones as intermediates, has been found to be of wide application in the preparation of natural products.[3–7]

A versatile method of preparing 1,3-diols is by reduction of β-hydroxyketones, synthesized from corresponding silyl enol ethers and aldehydes through Mukaiyama aldol reaction.[8] There are a number of methods described in the literature for the preparation of *syn*-1,3-diols[9] while the Tishchenko reduction[10] produces *anti*-diastereoisomers.

2. Results and discussion

In our attempts to obtain all the four isomers of enantiomerically enriched 1-phenyl-1,3-butanediols we

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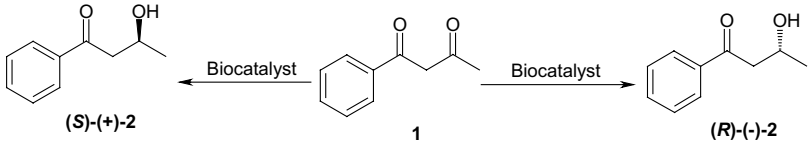
doi:10.1016/j.tetasy.2004.04.022

envisaged to initially select a suitable biocatalyst for the preparation of intermediate 3-hydroxy-1-phenyl-1-butanone in enantiomerically pure form. Consequently, screening of several oxidoreductases from our culture collection as well as commercial sources was undertaken. In this study, 15 enzyme strains in the families of yeasts, bacilli and fungi were shortlisted by preliminary screening of reductase activity and among them nine were found to be effective in reducing the substrate **1**. In the first step, as reported in literature,[1b,11,12] bioreduction of 1-phenyl-1,3-butadione with *Saccharomyces cerevisiae* furnished a monohydroxy ketone (+)-**2** as the product, comprising predominantly the (*S*)-enantiomer of high enantiopurity, while *Geotrichum candidum* and *Aspergillus niger* are reported to furnish the (*R*)-enantiomer.[13] In our experiments, which include yeast, bacilli and fungi, bioreduction was carried out using resting cells and a bubbler was used to maintain anaerobic conditions at 30 °C. In all the bioreduction reactions of the substrate **1**, a single product (3*R*)- or (3*S*)-hydroxy-1-phenyl-1-butanone (Scheme 1) with varying degree of enantiomeric excess was obtained. In these transformations, the 3-oxo functional group adjacent to terminal methyl was stereoselectively reduced by the biocatalysts in moderate to excellent ee's leaving the other keto group at C-1 intact. *Bacillus pseudomegatherium* (RRL Acc. No: RRLB 008), *Candida crusei* (RRLY 003), *Pichia capsulata* (RRLY 002), *Physarium policephalum* (RRLF 005) and *S. cerevisiae* (RRLY 007) furnished (*S*)-**2**, with the best selectivity being displayed by *P. capsulata* (ee ~ 99%) followed by *Candida crusei* (ee ~ 94%). Bioreductions using *Arthrobacter crystallopoietis* (RRLB 281), *Pichia farinosa* (RRLY 004), *Pichia pseudopastoris* (RRLY 001) and *Zygosaccharomyces rouxii* (RRLY 006), furnished predominantly (*R*)-**2** and *Z. rouxii* furnished practically an enantiopure product (ee ~ 99%) followed by *Arthrobacter crystallopoietis* (ee ~ 83%). Surprisingly in all the bioreductions only the 3-oxo functional group was reduced, displaying complete regioselectivity and even after prolonged reaction time (14 days) there was no formation of the dihydroxy product that is, 1-phenyl-1,3-butanediol. The results of these experiments are summarized in Table 1.

The data in Table 1 clearly demonstrates the efficacy of *Z. rouxii* and *P. capsulata*, displaying excellent regioselectivity to produce enantiomers (−)-**2** and (+)-**2** that is (*R*)-(−)- and (*S*)-(+)-3-hydroxy-1-phenyl-1-butanone, respectively, in high enantiomeric excess and chemical yields. After the completion of the first step of biocatalytic reductions, addition of a chemical reducing agent that is, sodium borohydride or sodium cyanoborohydride in the same reaction pot comprising the biocatalyst in aqueous medium at 5 °C, resulted in smooth transformation to optically active 1-phenyl-1,3-butanediols (−)-**4** and (+)-**4**, respectively, in high yields. Scheme 2 represents the best results obtained in one-pot enzymochemical reduction reaction using yeast strains namely *P. capsulata* and *Z. rouxii*.

The diastereoisomeric ratio (de) of *syn*- and *anti*-1-phenyl-1,3-butanediols as determined by proton NMR and total yields of the final products are shown in Table 2.[14] The results demonstrate that the one-pot sodium borohydride reduction in the presence of biocatalysts such as *P. capsulata* and *Z. rouxii*, displayed improved diastereoselectivity, resulting in the formation of *anti* products (de ~ 98%) while sodium cyanoborohydride displayed comparatively lower diastereoselectivity (de ~ 66–76%). The possibility of any influence exerted by the presence of glucose on the diastereomeric ratio was ruled out when experiments were run with biocatalyst with or without using 5% glucose in which the de remained unaffected.



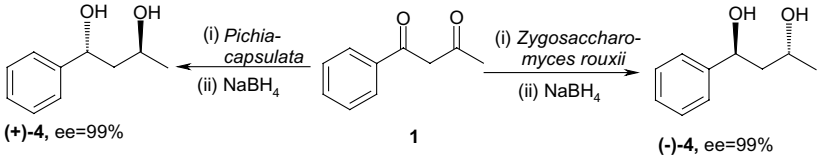
Scheme 1.

Table 1. Bioreduction of 1-phenyl-1,3-butanedione **1** with micro-organisms

Entry	Micro-organism	Yield (%)	Ee (%)[a]	Reaction time (h)	Absolute configuration[b]	$[\alpha]_D^{25}$ $CHCl_3$
1	*Bacillus pseudomegatherium*	50	46	72	*S*	+31.5
2	*Candida crusei*	82	94	48	*S*	+64
3	*P. capsulata*	85	99	18	*S*	+67.5
4	*Physarium policephalum*	60	53	72	S	+36
5	*S. cerevisiae*	80	77	72	*S*	+48.5
6	*Arthrobacter crystallopoietis*	75	83	48	*R*	−56.4
7	*Pichia farinosa*	70	67	48	*R*	−45.5
8	*Pichia-pseudopastoris*	80	78	48	*R*	−49
9	*Zygosaccharomyces rouxii*	85	99	40	*R*	−67.5

[a] The ees were determined by Chiral HPLC (Chiralcel OD-H, mobile phase; hexane–isopropanol = 95:5) analyzed after isolation of the bioproducts by column chromatography. Retention times of (*R*)- and (*S*)-enantiomers are 15 and 16.5 min, respectively.

[b] The absolute configurations were assigned by comparison of the sign of the specific rotation with that reported in the literature.[1b,11]



Scheme 2.

Table 2. One-pot enzymo-chemical reduction of **1** to (−)-**4** and (+)-**4**

Micro-organism	Reaction time for the formation of (−)-**2**/(+)-**2**	Ee (%) (conf.) of (−)-**2**/(+)-**2**	*syn–anti*[c]	*anti*-Isomer ee% (conf.)[d]	*syn*-Isomer ee% (conf.)[d]	Total yield (%)
P. capsulata	24	99 (*S*)[a]	1:99	99 (1*R*,3*S*)	ND	70
P. capsulata	24	99 (*S*)[b]	1:99	99 (1*R*,3*S*)	ND	68
P. capsulata	30	99 (*S*)[a]	12:88	99 (1*R*,3*S*)	99 (1*S*,3*S*)	67
P. capsulata	30	99 (*S*)[b]	13:87	99 (1*R*,3*S*)	99 (1*S*,3*S*)	65
Z. rouxii	40	99 (*R*)[a]	1:99	99 (1*S*,3*R*)	ND	75
Z. rouxii	40	99 (*R*)[b]	1:99	99 (1*S*,3*R*)	ND	72
S. cerevisiae	60	77 (*S*)[a]	15:85	75 (1*R*,3*S*)	75 (1*S*,3*S*)	60
S. cerevisiae	60	77 (*S*)[b]	17:83	75 (1*R*,3*S*)	75 (1*S*,3*S*)	60
S. cerevisiae	60	77 (*S*)[b]	20:80	71 (1*R*,3*S*)	73 (1*S*,3*S*)	62
B. gatherium	72	47 (*S*)[b]	1:99	41 (1*R*,3*S*)	ND	55

ND—not determined.
[a] $NaBH_4/NaCNBH_3$ was used in presence of enzyme without nutrient.
[b] $NaBH_4/NaCNBH_3$ was used in presence of enzyme and 5% glucose solution.
[c] *syn–anti* Ratio determined by proton NMR.
[d] Ee% determined by Chiral HPLC. The ee% of (−)-**2** and (+)-**2** was determined by analyzing the aliquots after the completion of the reaction as monitored by TLC.

To assess the role of the biocatalysts in influencing the diastereoselectivity in the formation of 1,3-diols, chemoreduction of bioreduced products (−)-**2** and (+)-**2**, purified by column chromatography was carried out in the absence of biocatalysts. The reducing agents used are lithium aluminium hydride in dry THF/diethyl ether at 0–5 °C (Table 3, entries 1–3), sodium borohydride (Table 3, entries 4–7), sodium cyanoborohydride (Table 3, entries 8–11) (with or without the presence of glucose) (Scheme 3).

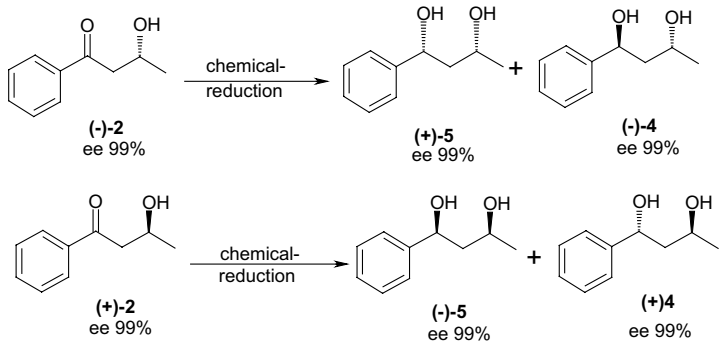
The reduction of (−)-**2** and (+)-**2** with sodium borohydride and sodium cyanoborohydride afforded almost equimolar ratio of enantiomerically pure *syn*- and *anti*-diols (42:58) and (50:50), respectively, which could easily be separated by column chromatography. However, reduction with LAH in dry diethyl ether showed some improvement in the diastereoselectivity, resulting in the formation of *syn*- and *anti*-diols in 73:27 ratio (Table 3).

From above studies it appears that the presence of the biocatalyst in the reaction medium does play an important role in guiding the diastereoselectivity of the reduced products during chemical reductions. Whether the crude enzyme acts as a solid support or matrix during the transformations or the chemical reducing agent forms a weak complex with the biocatalyst is not clear. It may also be possible that the cell surface and not the enzyme (protein) helps in modifying the diastereoselectivity of the final product. To rule out the possibility

Table 3. Chemical reduction of 3-hydroxy-1-phenyl-1-butanone (−)-**2** and (+)-**2** after eliminating the enzyme

Entry	Substrate	Reagent	Solvent	Product ratio *syn–anti*[b]	Conf. *syn* diol–*anti* diol	Total yield (%)
1	(−)-**2**	$LiAlH_4$	Ether[a]	73:27	(1*R*,3*R*):(1*S*,3*R*)	90
2	(−)-**2**	$LiAlH_4$	THF[a]	74:26	(1*R*,3*R*):(1*S*,3*R*)	86
3	(+)-**2**	$LiAlH_4$	Ether[a]	72:28	(1*S*,3*S*):(1*R*,3*S*)	87
4	(−)-**2**	$NaBH_4$	MeOH[a]	42:58	(1*R*,3*R*):(1*S*,3*R*)	85
5	(−)-**2**	$NaBH_4$	H_2O[c]	50:50	(1*R*,3*R*):(1*S*,3*R*)	85
6	(−)-**2**	$NaBH_4$	H_2O[d]	45:55	(1*R*,3*R*):(1*S*,3*R*)	75
7	(+)-**2**	$NaBH_4$	H_2O[c]	50:50	(1*S*,3*S*):(1*R*,3*S*)	85
8	(−)-**2**	$NaCNBH_3$	MeOH[a]	50:50	(1*R*,3*R*):(1*S*,3*R*)	86
9	(−)-**2**	$NaCNBH_3$	H_2O[c]	30:70	(1*R*,3*R*):(1*S*,3*R*)	78
10	(+)-**2**	$NaCNBH_3$	H_2O[c]	28:72	(1*S*,3*S*):(1*R*,3*S*)	75
11	(+)-**2**	$NaCNBH_3$	MeOH[a]	50:50	(1*S*,3*S*):(1*R*,3*S*)	82

[a] Reactions performed at 0–5 °C.
[b] *syn–anti* Ratio was determined by ^1H NMR.
[c] Reaction performed at room temp (20–25 °C).
[d] Reaction performed at room temp in 5% glucose solution.

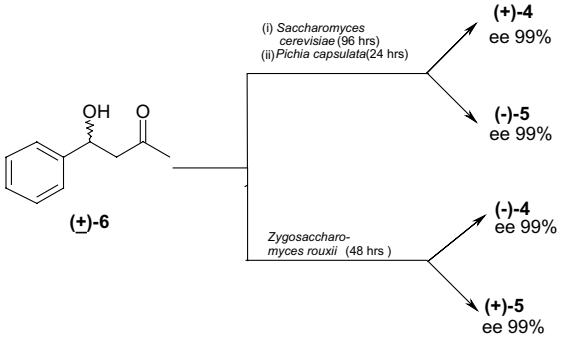


Scheme 3.

of the cell surface playing any role we also carried out the one-pot chemical reductions of (−)-**2** and (+)-**2** with sodium borohydride in the presence of a cell free extract of biocatalysts. The final results in these experiments matched with those obtained in presence of whole cells (Table 2) as again formation of predominantly *anti* products was observed. These experiments clearly demonstrate that it is possible to carry out one-pot sequential transformations advantageously without the need to isolate the intermediates or sacrifice overall yields of the final products.

The specific rotation values and the enantiomeric excess of all the four diastereomers thus prepared along with the reported ones in literature[15,16] are given in the Table 4. It should be emphasized here that the present strategy of one-pot enzymo-chemical reductions to prepare the enantiomerically enriched *syn*- and *anti*-1-phenyl-1,3-butanediols seems to be better than those reported earlier so far as the yields and enantiopurity of (−)-**4** and (+)-**4** are concerned.

As our attempts to reduce both the keto groups of the substrate **1** by successive bioreductions using a single biocatalyst to prepare all of the possible enantiopure isomers of 1-phenyl-1,3-diols through the intermediates (−)-**2** and (+)-**2** did not succeed, we therefore envisaged bioreduction of another intermediate that is, (±)-4-hydroxy-4-phenyl-butan-2-one **6** (prepared by catalytic hydrogenation of **1** in 84% yield) as an alternative route to the formation of enantiopure 1,3-diols. Thus (±)-**6** was subjected to bioreduction (Scheme 4) using *P. capsulata* and *S. cerevisiae,* and, as expected, the biocatalytic reduction was facile with *P. capsulata* affording a high enantiomeric excess (99%) and isolated yields (75%) with an equal ratio of both the diastereoisomers (+)-**4** and (−)-**5**. *S. cerevisiae* on the other hand afforded predominantly the *anti*-diastereoisomer of high enantiomeric excess (ee ~ 99%) though the isolated yields were low (45–50%).



Scheme 4.

Bioreduction of (±)-**6** using *Z. rouxii* was also carried out in a diastereospecific manner though with reversal of selectivity, thereby giving other pair of enantiomerically pure diastereoisomers (−)-**4** and (+)-**5** in good isolated yields (~70%). The results of these experiments are summarized in Table 5.

3. Conclusion

All the four diastereoisomers of 1-phenyl-1,3-butanediols were successfully prepared from corresponding 1-phenyl-1,3-butadione, applying chemo-enzymatic and enzymo-chemical methodologies using a number of reductases, best among them are *Z. rouxii*, *S. cerevisiae* and *P. capsulata*. It was also established conclusively that the presence of enzymes in the reaction media imparts an important role in influencing the diastereoselectivity of the products during one-pot enzymo-chemical transformations. Yields of the final products

Table 4. Specific rotation values of isolated bioproducts (diols)

Entry	Compound	Conf.	Ee (%)	$[\alpha]_D^{25}$ (Concn)	Ee (%) lit.[14,15]	$[\alpha]_D^{25*}$ (Concn)
1	(−)-**4**	(1*S*,3*R*)	99	−61.6 (0.7)	94	−65.0 (3.4)
2	(+)-**4**	(1*R*,3*S*)	99	+61.0 (1.0)	98	+61.1 (3.4)
3	(−)-**5**	(1*S*,3*S*)	99	−58.7 (1.2)	98	−48.9 (0.8)
4	(+)-**5**	(1*R*,3*R*)	99	+56.5 (2.2)	98	+49.4 (0.8)

* Literature value.[15,16]

Table 5. Preparation of (−)-**4**, (+)-**4**, (+)-**5** and (−)-**5** by the biotransformation of (±)-**6**

Substrate	Micro-organism	Reaction time (h)	Product	Yield (%)	Conf.	Ee (%)	$[\alpha]_D^{25}$
(±)-**6**	*P. capsulata*	24	(+)-**4**	38	(1*R*,3*S*)	99	+61.0
			(−)-**5**	38	(1*S*,3*S*)	99	−58.7
(±)-**6**	*S. cerevisiae*	96	(+)-**4**	30	(1*R*,3*S*)	99	+61.0
			(−)-**5**	15	(1*S*,3*S*)	99	−55.0
(±)-**6**	*Z. rouxii*	48	(−)-**4**	23	(1*S*,3*R*)	99	−61.6
			(+)-**5**	46	(1*R*,3*R*)	99	+56.5

are much higher in one-pot transformations than in two step sequential reactions.

4. Experimental

4.1. General

^{1}H NMR spectra were recorded as δ values at 200 MHz NMR and ^{13}C NMR at 50 MHz using $CDCl_3$ as a solvent and TMS as internal standard. Infrared spectra were recorded as KBr pellets in cm^{-1} on a Hitachi 270-30 model spectrophotometer. Mass spectra were recorded on JEOL MSD-300 mass spectrophotometer. Optical rotations were measured on Perkin–Elmer 241 polarimeter in $CHCl_3$ solution. Chiral HPLC was carried out on a Shimadzu LC-10 AT model. HPLC analysis of 1-phenyl-3-hydroxy-butan-1-one was performed on Chiralcel OD-H (chiral column), using isopropanol–hexane = 5:95 as eluent, flow rate 0.8 mL/min where as the HPLC analysis of 1,3-diols was carried out on (*R*,*R*)-Whelk-O1, using hexane–isopropanol–acetic acid = 96.9:3.0:0.1 as eluent, flow rate 0.5 mL/min.

4.2. Cultivation of microorganisms

For the general preparation of biomass the following solution was used ($g\,L^{-1}$); yeast 3 g, dextrose 20 g, peptone 10 $g\,L^{-1}$, pH adjusted to 6.5. The medium autoclaved at 120 °C for 20 min. The dextrose solution autoclaved separately and used prior to inoculation. Flask (1000 mL) filled with 250 mL of the medium inoculated and cultivated under CO_2 atmosphere on a rotary shaker (200–220 rpm), temp 30 °C. The optical density (OD) of the medium was observed at 610 nm and the medium centrifuged at 8000 rpm with temp at 10–15 °C for 8–10 min. The pellet washed with autoclaved distilled water and centrifuged again. The cell pellet thus obtained is used as such for biotransformation.

4.3. General method for the biocatalytic reduction of benzoylacetone

In a typical experiment, to a suspension of *Z. rouxii* in distilled water (7.5 g wet pellet in 75 mL) containing glucose (5 g) was added ethanolic solution of compound **1** (162 mg, 1.0 mmol, 0.3 mL) and the contents were shaken at 30 °C as such using a bubbler. After completion of the reaction (40 h), the contents were centrifuged and the supernatant and cell pellet were extracted separately with diethyl ether (3 × 70 mL). The combined organic layer was washed with water, dried over anhydrous sodium sulfate and concentrated under reduced pressure to furnish the crude product, which on purification by column chromatography on silica gel and elution with hexane–ethyl acetate (85:15) afforded (*R*)-3-hydroxy-1-phenyl-butan-1-one (−)-**2** as a colourless oil (140 mg, 85%). $[\alpha]_D^{25} = -67.5$ (*c* 1.2, $CHCl_3$) 99% ee (by Chiral HPLC analysis.); IR (KBr): 3423, 2970, 1681, 1449, 1374, 1003, 754, 690 cm^{-1}; ^{1}HNMR (200 MHz, $CDCl_3$): δ 1.30 (3H, d, $J = 6.4$ Hz, CH_3), 3.10 (2H, d, $J = 6.0$ Hz, CH_2), 3.24 (1H, OH), 4.42 (1H, m, CHOH), 7.50 (3H, m, ArH), 7.97 (2H, dd, $J = 2.0$, 8.5 Hz, ArH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 22.47, 46.54, 64.08, 128.12, 128.75, 133.63, 136.75, 200.95; MS *m*/*z* (%): 164 (2.4), 146 (11.4), 120 (12.4), 106 (100), 91 (5), 77 (60), 52 (32). Anal. Calcd for $C_{10}H_{12}O_2$: C, 73.17; H, 7.31. Found: C, 73.55; H, 7.39.

4.4. Bioreduction with *S. cerevisiae*

To a stirring solution of glucose (8 g) and *S. cerevisiae* (13 g) in distilled water (100 mL) was added ethanolic solution of compound **1** (540 mg, 3.33 mmol, 1.0 mL) and the contents stirred for 72 h at 30 °C. The mixture was worked up as described in Section 4.4 to furnish crude bioproduct (+)-**2**, which on purification by column chromatography on silica gel with ethyl acetate–*n*-hexane (15:85) as eluant gave (+)-**2** (440 mg, 80%). $[\alpha]_D^{25} = +48.5$ (*c* 2.8, $CHCl_3$) 77% ee (HPLC analysis); IR (KBr): 3427, 2971, 1681, 1449, 1373, 1002, 754, 690 cm^{-1}.

4.5. Synthesis of racemic *syn*- and *anti*-1-phenyl-1,3-butanediol; (±)-4 and (±)-5

Compound **1** (6.48 g, 40 mmol) was reacted with sodium borohydride (2 g, 52.9 mmol) in methanol (110 mL) at 0–5 °C to afford a mixture of *syn*- and *anti*-1-phenyl-1,3-butanediol [(±)-**5** and (±)-**4**, 6.0 g, 90% yield] after usual workup. The diol mixture was divided into two parts. The first part was processed for the isolation and separation of racemic **4** and **5** and second part was used for the preparation of (±)-**2**. Column chromatography of part one on silica gel and elution with ethyl acetate–*n*-hexane (20:80) affords (±)-**5** (*syn* product as a semisolid (900 mg, 27%). IR (KBr): 3260, 3027, 2967, 2866, 1601, 1491, 1452, 1373, 1321, 1133, 1073, 974, 760, 700 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.23 (3H, d, $J = 6.2$ Hz, CH_3), 1.81 (2H, m, CH_2), 4.15 (1H, m, $CHCH_3$), 4.94

(1H, dd, $J = 3.54$, 9.55 Hz, ArCH) and 7.31 (5H, m, ArH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 23.90, 46.91, 68.86, 75.20, 125.67, 127.50, 128.43, 144.50; MS m/z (%) 166 (4.3), 148 (25.5), 133 (9.5), 107 (100), 105 (66.8), 104 (17.8), 79 (89.5), 77 (54.8). Anal. Calcd for $C_{10}H_{14}O_2$: C, 72.28; H, 8.43. Found: C, 72.82; H, 8.50. Further elution with the same eluent gave racemic (±)-**4** as a crystalline solid mp 80 °C (2.10 g, 63%). IR (KBr): 3395, 3308, 2972, 1586, 1494, 1455, 1423, 1378, 1350, 1333, 1319, 1129, 1049, 969, 762, 704, 548 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): 1.20 (3H, d, $J = 6.2$ Hz, CH_3), 1.84 (2H, m, CH_2), 4.03 (1H, m, $CHCH_3$), 5.01 (1H, dd, $J = 4.6$, 6.5 Hz, ArCH) and 7.31 (5H, m, ArH). ^{13}C NMR (50 MHz $CDCl_3$): δ 23.50, 46.17, 65.36, 71.71, 125.63, 127.34, 128.46, 144.50; MS m/z (%): 166 (3.9), 148 (23), 133 (11), 108 (11.0), 107 (100), 105 (72), 104 (18.3), 91 (9.3), 79 (99), 77 (74). Anal. Calcd for $C_{10}H_{14}O_2$: C, 72.28; H, 8.43. Found: C, 72.61; H, 8.47.

4.6. Synthesis of (±)-3-hydroxy-1-phenyl-butanone (±)-2

A mixture of (±)-**4** and (±)-**5** (1.39 g, 8.4 mmol) was reacted with pyridinium dichromate (3.15 g, 8.4 mmol) in dimethylformamide (8 mL), the contents stirred at 0 °C for 2.5 h and the reaction was monitored by TLC. After completion of the reaction, the mixture was diluted with diethyl ether (150 mL), filtered and the solid washed with diethyl ether (2 × 50 mL) and the combined organic layer was concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel to give (±)-**2** as an oil (760 mg, 55%). IR (KBr): 3423, 2971, 1680, 1449, 1374, 1002, 754, 690 cm^{-1}. IR of (±)-**2** was super-imposable with that of (−)-**2**.

4.7. One-pot preparation of (−)-4

To a suspension of *Z. rouxii* (wet pellet, 6.5 g) in distilled water (65 mL) containing glucose (4 g) was added ethanolic solution of compound **1** (100 mg, 0.61 mmol, 1 mL) and the contents were shaken at 28 °C using a bubbler. The reaction was monitored by TLC, after the completion of the reaction (40 h), the ee% of the intermediate (−)-**2** was determined by taking out 5 mL test sample and analyzing it by Chiral HPLC using Chiralcel OD-H. Bubbler was removed and the contents cooled to 15 °C and to the stirring solution was added $NaBH_4$ (25 mg, 0.64 mmol) in three small portions. After the completion of the reaction (2 h), workup and HPLC analysis of the final product showed the diastereoisomeric excess of 98% and ee of >99%. Purification of the crude product by column chromatography on silica gel afforded sole product (−)-**4** (75 mg, 75%). $[\alpha]_D^{25} = -61.6$ (c 0.7, $CHCl_3$) ee 99%; IR (KBr): 3405, 3030, 2970, 2928, 1603, 1494, 1454, 1376, 1334, 1289, 1237, 1208, 1135, 1048, 974, 908, 809, 700, 665 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.19 (3H, d, $J = 6.3$ Hz, CH_3), 1.83 (2H, m, CH_2), 4.02 (1H, m, $CHCH_3$), 4.99 (1H, dd, $J = 4.5$, 6.8 Hz, Ar–CH) and 7.28 (5H, m, ArH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 23.36, 46.21, 65.14, 71.43, 125.59, 127.19, 128.35, 144.48; MS m/z (%) 166 (4.1), 148 (26.9), 133 (12), 107 (96.4), 105 (68.1), 79 (100), 77 (72). Anal. Calcd for $C_{10}H_{14}O_2$: C, 72.28; H, 8.43. Found: C, 72.66; H, 8.51.

4.8. One-pot synthesis of (+)-4

To a suspension of *P. capsulata* (wet pellet, 6.5 g) in distilled water (65 mL) containing glucose (4 g) was added ethanolic solution of compound **1** (100 mg, 0.61 mmol, 1 mL) and the contents shaken at 28 °C using a bubbler. Reaction was monitored by TLC, the ee% and the configuration of the intermediate (+)-**2** was determined by taking out 5 mL test sample, extracting it with diethylether, followed by desolventization and analysis on Chiral HPLC using Chiracel OD-H. Bubbler was removed and the contents were cooled to 15 °C and to the stirring solution was added $NaBH_4$ (25 mg, 0.64 mmol) in three small portions. After the completion of the reaction, HPLC analysis showed de of 98% and ee of 99%. Purification of the bioproduct by column chromatography on silica gel gave (+)-**4** (70 mg, 70%) $[\alpha]_D^{25} = +61$ (c 1.0, $CHCl_3$); 99% ee; IR (KBr): 3355, 3030, 2968, 2928, 1607, 1492, 1452, 1404, 1336, 1129, 1054, 974, 755, 700, 551 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.18 (3H, d, $J = 6.2$ Hz, CH_3), 1.81(2H, m, CH_2), 4.01 (1H, m, $CHCH_3$), 4.98 (1H, dd, $J = 6.6$, $J = 3.7$ Hz Ar–CH) and 7.29 (5H, m, ArH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 23.36, 46.26, 65.11, 71.39, 125.59, 127.23, 128.37, 144.46; MS m/z (%) 166 (4.2), 148 (28.4), 133 (10), 107 (98.9), 105 (71.8), 79 (83.2), 77 (59.6), 56 (10.4), 52 (30.4), 47 (30.4). Anal. Calcd for $C_{10}H_{14}O_2$: C, 72.28; H, 8.43. Found: C, 72.62; H, 8.41.

4.9. $LiAlH_4$ reduction of (−)-2: preparation of (−)-4 and (+)-5

To a solution of bioproduct (−)-**2** (328 mg, 2.0 mmol) in diethyl ether (70 mL), LAH (57 mg, 1.5 mmol) was added in three installments at 0 °C and the reaction was monitored by TLC. After the completion of the reaction, the contents were worked up by quenching the unreacted LAH by the addition of ethyl acetate (50 mL) followed by addition of water (30 mL), separation of the organic layer followed by drying over anhydrous sodium sulfate and concentration in vacuo gave the crude mixture of (−)-**4** and (+)-**5**. The products (−)-**4** and (+)-**5** were separated by column chromatography over silica gel using the same mixture of solvents as eluent as described for the separation of racemic (±)-**4** and (±)-**5** to give (−)-**4** as a semisolid (72 mg, 22%) and (+)-**5** as a solid mp 65 °C (225 mg, 68%); IR (KBr): 3401, 3254, 3026, 2966, 2926, 2865, 1637, 1492, 1453, 1378, 1322, 1258, 1198, 1134, 1070, 934, 759, 699 cm^{-1}. ^{1}H NMR (200 MHz., $CDCl_3$): δ 1.19 (3H, d, $J = 6.0$ Hz, CH_3), 1.82 (2H, m, CH_2), 4.09 (1H, m, $CHCH_3$), 4.89 (1H, dd, $J = 3.0$, 9.4 Hz, ArCH), 7.29 (5H, m, ArH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 23.97, 46.91, 68.76, 75.15, 125.67, 127.53, 128.45, 144.47; MS m/z (%): 166 (3.9), 148 (23), 133 (11), 107 (100), 105 (72), 79 (99), 77 (74). Anal. Calcd for $C_{10}H_{14}O_2$:C, 72.28; H, 8.43. Found: C, 72.42; H, 8.45.

4.10. $NaCNBH_3$ reduction of (−)-2: preparation of (−)-4 and (+)-5

Bioproduct (−)-**2** (164 mg, 1.0 mmol) and sodium cyanoborohydride (126 mg, 2.0 mmol) were dissolved in methanol (8 mL) at 0 °C, a trace of bromocresol Green was also added to monitor the reaction. The solution was immediately turned to blue and 3 N HCl methanol was added dropwise to restore the yellow colour. The solution was stirred for 2 h and the contents were concentrated at reduced pressure and the residue was extracted with diethyl ether (3 × 20 mL). The combined extracts were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The mixture was separated by column chromatography on silica gel and eluted with ethyl acetate–*n*-hexane; 20:80 to give (+)-**5** as a solid (70 mg, 43%). $[\alpha]_D^{25} = +52.5$ (*c* 1.2, $CHCl_3$) 99% ee; and (−)-**4** as a semisolid (70 mg, 43%). $[\alpha]_D^{25} = -61.6$ (*c* 0.7, $CHCl_3$) 99% ee.

4.11. $NaBH_4$ reduction of (−)-2: preparation of (−)-4 and (+)-5

To a solution of (−)-**2** (113 mg, 0.70 mmol) in methanol (50 mL), $NaBH_4$ (19 mg, 0.5 mmol) was added at 0 °C and the reaction was monitored by TLC. After the completion of the reaction, the reaction was worked up as usual and the products were separated by column chromatography on silica gel as described for LAH reduction to give (+)-**5** as a solid (40 mg, 35%) $[\alpha]_D^{25} = +52.5$ (*c* 1.2, $CHCl_3$) 99% ee and (−)-**4** as a semisolid (55 mg, 48%). $[\alpha]_D^{25} = -61.6$ (*c* 0.7, $CHCl_3$) 99% ee.

4.12. $NaCNBH_3$ reduction of (+)-2: preparation of (+)-4 and (−)-5

Bioproduct (+)-**2** (492 mg, 3.0 mmol) in methanol (8 mL) and sodium cyanoborohydride (440 mg, 7.0 mmol) was made to react in the presence of bromocresol Green (1 mg to monitor the reaction) at 0 °C and the reaction was worked up as described for the preparation of (−)-**4** and (+)-**5**, to give after column chromatography on silica gel (−)-**5** (206 mg, 41%) $[\alpha]_D^{25} = -49$ (*c* 0.8, $CHCl_3$) 94% ee; IR (KBr): 3254, 3026, 2967, 2929, 2865, 1603, 1491, 1453, 1377, 1321, 1134, 1071, 974, 759 and 700 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.19 (3H, d, $J = 6.1$ Hz, CH_3), 1.78 (2H, m, CH_2), 4.09 (1H, m, $CHCH_3$), 4.87 (1H, dd, $J = 3.4$, 3.3 Hz, ArCH) and 7.30 (5H, m, ArH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 23.98, 46.91, 68.76, 75.15, 125.70, 127.55, 128.47, 144.50; MS *m*/*z* (%) 166 (4.3), 148 (25.3), 107 (99), 105 (66), 79 (88), 77 (54.8), 56 (8.6), 52 (30.4), 47 (30.4). Anal. Calcd for $C_{10}H_{14}O_2$:C, 72.28; H, 8.43. Found: C, 72.12; H, 8.48. (+)-**4** (206 mg, 41%) $[\alpha]_D^{25} = +58$ (*c* 0.8, $CHCl_3$) 94% ee.

4.13. $LiAlH_4$ reduction of (+)-2: preparation of (+)-4 and (−)-5

To a solution of (+)-**2** (164 mg, 1.0 mmol) in diethyl ether (50 mL), LAH (38 mg, 1.0 mmol) was added in three installments at 0 °C and the reaction was monitored by TLC. After the completion of the reaction the contents were worked up as described for the preparation of (−)-**4** and (+)-**5** to give after purification by column chromatography on silica gel (−)-**5** as a solid, mp 65 °C (104 mg, 63%) $[\alpha]_D^{25} = -52$ (*c* 0.8, $CHCl_3$) 94% ee and (+)-**4** as a semisolid (40 mg, 24%). $[\alpha]_D^{25} = +58$ (*c* 0.8, $CHCl_3$) 94% ee.

4.14. Synthesis of racemic 4-hydroxy-4-phenyl-butan-1-one (±)-6

To a solution of **1** (11.340 g, 70.0 mmol) in ethanol (80 mL) was added 10% Pd/C (650 mg) and the contents hydrogenated at 45 psi. The reaction was monitored by TLC. After usual workup, the crude product was purified by column chromatography on silica gel to give racemic ketol (±)-**6** (9.53 g, 86%). IR (KBr): 3419, 2919, 1709, 1493, 1361, 1162, 1060, 755, 700 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 2.17 (3H, s, CH_3), 2.83 (2H, d, $J = 6.5$ Hz, $COCH_2$), 3.05 (1H, s, exchangeable with D_2O), 5.17 (1H, d, $J = 6.5$ Hz, CHOH), 7.36 (5H, s, ArH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 30.79, 52.04, 69.85, 125.65, 127.66, 128.53, 142.88, 209.04; MS *m*/*z* (%): 164 (100) M^+, 146 (94), 131 (37), 107 (99.2), 105 (99.1), 91 (5), 79 (99.1), 77 (60), 58 (97.7). Anal. Calcd for $C_{10}H_{12}O_2$: C, 73.17; H, 7.31. Found: C, 73.42; H, 7.41 and a mixture of (±)-**4** and (±)-**5** (440 mg, 2.5%).

4.15. Bioreduction of (±)-6 with *Z. rouxii*: preparation of (−)-4 and (+)-5

Wet pellet of *Z. rouxii* (12 g) was suspended in a flask containing glucose (7 g) and distilled water (120 mL) and solution of compound **6** (328 mg, 2.0 mmol) in ethanol (1 mL) was added to it. The contents were stirred for 48 h at 30 °C using a bubbler. Workup of the reaction mixture furnished a crude product. Purification by column chromatography on silica gel gave (+)-**5** (150 mg, 45%); $[\alpha]_D^{25} = +52.5$ (*c* 0.9, $CHCl_3$) >99% ee (HPLC) and (−)-**4** (72 mg, 22%), $[\alpha]_D^{25} = -61.6$ (*c* 0.7, $CHCl_3$), >99% ee.

4.16. Bioreduction of (±)-6 with *P. capsulata*: preparation of (+)-4 and (−)-5

Racemic **6** (100 mg, 0.61 mmol) in ethanol (1 mL) was subjected to bioreduction with *P. capsulata* (6.5 g wet pellet, 65 mL distilled water and 5 g glucose) at 25 °C. The reaction was worked up after 24 h. Purification by column chromatography on silica gel and elution with ethyl acetate–*n*-hexane (20:80) gave (+)-**4** as a semisolid (38 mg), $[\alpha]_D^{25} = +61$ (*c* 0.9, $CHCl_3$) 99% ee and (−)-**5** as a solid (38 mg), $[\alpha]_D^{25} = -58.7$ (*c* 0.5, $CHCl_3$) 99% ee.

4.17. Bioreduction of (±)-6 with *S. cerevisiae*: preparation of (+)-4 and (−)-5

A solution of racemic **6** (100 mg, 0.61 mmol) in ethanol (1 mL) was subjected to bioreduction with *S. cerevisiae*

(8 g, distilled water 60 mL, glucose 4 g) at 25 °C and worked up after 96 h and after purification by column chromatography on silica gel, and elution with ethyl acetate–*n*-hexane (20:80) to afford (+)-**4**, (30 mg, 30%), $[\alpha]_D^{25} = +61$ (*c* 1.0, $CHCl_3$) 99% ee and (−)-**5** as a solid (15 mg, 15%), $[\alpha]_D^{25} = -55$ (*c* 1.2, $CHCl_3$) 99% ee.

References and notes

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14. The racemic *syn*- and *anti*-diols (as standards for HPLC and spectral analysis) were prepared by $NaBH_4$ reduction of compound **1** followed by column chromatography on silica gel giving (±)-**4** and (±)-**5**, respectively, in overall yield of 90%. The diastereoisomers (−)-**4** and (−)-**5**; (+)-**4** and (+)-**5** and the synthetic samples of (±)-**4** and (±)-**5** were analyzed by Chiral HPLC using (*R*,*R*)-Whelk-O1 column with mobile phase *n*-hexane–isopropanol–acetic acid (96.9:3.0:0.1).
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