Facile Biocatalytic Reduction of the Carbon–Carbon Double Bond of 5-Benzylidenethiazolidine-2,4-diones. Synthesis of (±)-5-(4-{2-[Methyl(2-pyridyl)amino]ethoxy}benzyl)thiazolidine-2,4-dione (BRL 49653), its (*R*)-(+)-Enantiomer and Analogues

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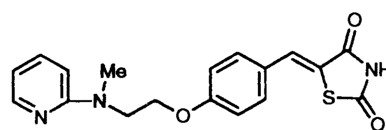
A novel biotransformation system for the reduction of carbon-carbon double bonds in 5-benzylidene-thiazolidine-2,4-diones, to give the corresponding 5-benzylthiazolidine-2,4-diones, using whole cells of red yeasts is described. These reduced compounds, which are recovered in good yield, are of potential use in the treatment of non-insulin dependent diabetes mellitus. The mild reaction conditions developed allow reduction of 5-benzylidenethiazolidine-2,4-diones containing other functionalities which are not compatible with alternative reduction methods. The biocatalytic reduction is enantioselective and the synthesis of *R*-(+)-5-(4-{2-[methyl(2-pyridyl)amino]ethoxy}benzyl)thiazolidine-2,4-dione by *Rhodotorula rubra* CBS 6469 and structure confirmation by X-ray crystallography is detailed. Optimisation of reaction conditions (including immobilisation) for these whole cell reduction systems is described.

Reductions of carbon-carbon double bonds catalysed by enzyme systems are not subject to the same constraints as those catalysed by non-biochemical processes. There is no requirement for elevated temperatures or pressures and the conversion is not prone to inhibition by the same elements or functionalities. There is, however, a requirement for the use and regeneration of the cofactors. The carbon-carbon double bond to be reduced usually needs to be activated by adjacent electron withdrawing functionality. For example, fermenting bakers' yeast reduces nitroalkenes to nitroalkanes[1] and also reduces 2-chlorobut-2-enoates, with concomitant hydrolysis, to the corresponding 2-chlorobutyric acids.[2] In many instances the carbon-carbon double bond reduction is associated with the reduction of other functional groups within the molecule. For example, in the microbial reduction of 3-fluoro-4-phenyl-1-(*p*-tolylsulfonyl)but-3-ene-2-one, a number of organisms showed greater activity in reducing the carbonyl group than the alkene.[3] There are reports of successful alkene reductions in the presence of carbonyl groups[4] although a mixture of products can often result. Selection of the correct organism, however, can give the desired alkane as the major product.[5] Stereocontrol in the reduction procedure is one of the major advantages of an enzyme catalysed reduction system[1-5] and can take the form of a stereoselective reduction of a prochiral molecule[6] or a stereospecific reduction of chiral entities.[7]

The study reported here is the search for a biocatalytic reduction of a prochiral carbon-carbon double bond in molecules containing other chemical functionalities which may be prone to reduction or hydrolysis. The initial target of this work was the reduction of the benzylidene compound **1**[8] to the benzylic compound **2** (BRL 49653) as, under non-biochemical catalytic reduction conditions, the additional presence of the pyridyl moiety in compound **1** necessitates the use of elevated temperatures and pressures. The biocatalytic reduction procedure identified has been extended to the synthesis of enantiomers and analogues of compound **2** which are not readily available by non-biochemical reductions. BRL 49653 and analogues are of potential use for the treatment of non-insulin dependent diabetes mellitus.[9,10]



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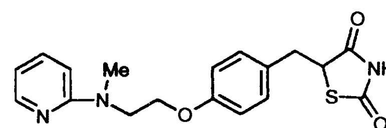


2, BRL 49653

Results and Discussion

Screening of a variety of yeasts (including *Candida*, *Saccharomyces* and *Pichia sp.*) involved incubation of the substrate **1** with whole cells of the organisms, which had been grown in a nutrient medium and then resuspended in a phosphate buffer (pH 8.0). Only the red yeasts gave significant conversion of the substrate to product after 24 h, although yields were low (Table 1).

The low levels of reduction were probably due, in part, to the insolubility of the substrate in the aqueous buffer systems. Two phase liquid–liquid systems or single liquid phase systems utilising a water miscible cosolvent can be employed in biotransformation studies to overcome such solubility problems.[11] The introduction of water immiscible solvents in whole cell reduction studies gave no discernible product formation in the presence of methyl acetate, ethyl acetate or diethyl malonate although a 20% v/v inclusion of ethyl acetoacetate into an

Table 1

Yeast	Conversion of compound **1** into compound **2** (%)[a]
Rhodotorula glutinis CBS 4406	13
Rhodotorula rubra CBS 17	9
Rhodotorula rubra CBS 6469	16
Rhodosporidium toruloides CBS 14	4

[a] Whole cell conversion of the alkene to the alkane over 24 h at pH 8.0, as measured by HPLC.

Table 2 Effect of 1,4-dioxan concentration on whole cell reductions by *Rhodotorula rubra* CBS 6469 in Tris–HCl pH 8.0 buffer

1,4-Dioxane-water content (% v/v)	Conversion of compound **1** into compound **2** (%)		
	2 h	4 h	6 h
4	23	24	26
8	48	51	54
12	58	61	60
16	43	51	46
20	21	11	12

incubation of cells of *Rhodotorula glutinis* CBS 4406 gave a 26% conversion of compound **1** to **2** over 24 h. Single liquid phase systems showed the best conversions using *Rhodotorula rubra* CBS 6469 in the presence of aqueous 1,4-dioxane. Substrate **1**, at 1 mg cm^{-3} concentration, was soluble in Tris–HCl buffer (pH 8.0) with > 16% v/v 1,4-dioxane; at lower organic solvent levels precipitation could be seen to occur, giving the solution a cloudy appearance. Incubations of whole cells of *Rhodotorula rubra* CBS 6469 in Tris–HCl buffer containing different levels of 1,4-dioxane were monitored for extent of conversion (Table 2). There was clearly a balance between complete solubility of the substrate in the system employed and the toxic effect of the organic solvent on the biotransformation system; 12% v/v aqueous 1,4-dioxane in buffer appears optimal.

Six hour incubations carried out in 12% v/v aqueous 1,4-dioxane showed little difference in the percentage reduction of substrate **1** to product **2** at pH 8.0 and 9.0, but higher conversions were achieved than at pH 7.0. This was probably due to the greater solubility of compound **1** in basic aqueous solvents than at neutral pH. Temperature optimisation studies in reactions at pH 8.0 with substrate (800 µg cm^{-3}) and aqueous 1,4-dioxane (12% v/v) showed an increase in reaction rate above 24 °C with both 28 and 30 °C showing comparable conversions.

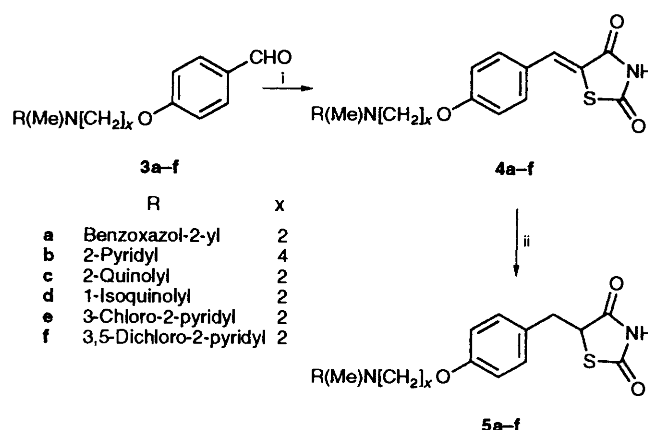
Successful reduction of the double bond in compound **1** was also demonstrated using alginate immobilised cells. The cells of *Rhodotorula rubra* CBS 6469 were immobilised in a buffer with 5% sucrose and the reduction of compound **1** by the immobilised cells was compared with that by an equal amount of free cells. Percentage conversions in the two systems showed no significant differences.

To establish the substrate specificity of this enzyme system, the reduction of related 5-benzylidenethiazolidine-2,4-diones **4a–f**, which were readily synthesised from their corresponding aldehydes **3** by condensation with thiazolidine-2,4-dione,[8,11] was studied (Scheme 1). In each case efficient reduction was achieved with *Rhodotorula rubra* CBS 6469 to give compounds **5a–f**. While compounds **5a–b** were also readily obtainable by non-biochemical reduction methods,[8] reduction with *Rhodotorula rubra* CBS 6469 proved effective with 5-benzylidenethiazolidine-2,4-diones where non-enzyme catalysed chemical reduction procedures proved unselective. Magnesium–methanol reduction[12] of the chloropyridyl compound **4e** resulted in complete dechlorination of the pyridine ring to give compound **2**.[13] Catalytic reduction [(H_2 (10%) Pd–C] led to either partial reduction of the (iso)quinoline ring, for substrates **4c** and **4d**, or to partial dechlorination of the pyridine ring for substrates **4e** and **4f**.[13] Using the whole cell red yeast system to effect the reduction gave compounds **5c–f** without the over reduction experienced with the chemical methods.

Table 3 Extent of reduction of *Rhodotorula rubra* CBS 6469 and the enantiomeric ratio[a]

Time (h)	Enantiomeric ratio	Product (%)
racemic standard	52:48	—
0.00	—:—	—
0.25	87:13	6.5
0.50	87:13	13
0.75	80:20	20
1.00	82:18	29
1.50	74:26	44
2.00	70:30	52
3.00	63:37	61
4.00	55:45	72
5.00	52:48	73

[a] Whole cell reactions at pH 8.0 with % conversion and enantiomer ratios measured by HPLC as described in the experimental section.



Scheme 1 *Reagents:* i, thiazolidine-2,4-dione; ii, *Rhodotorula rubra* CBS 6469

Reduction of the 5-benzylidenethiazolidine-2,4-diones **4b–d** was greater at pH 9.0 than at pH 8.0; again, this is probably due to the increased solubility of these substrates at the higher pH.

Generation of a chiral centre in the biocatalytic reduction presents the opportunity for the synthesis of an enantiomerically pure product, although it is known that 5-benzylthiazolidine-2,4-diones racemise rapidly.[14] The whole cell biotransformation of compound **1** to **2** was followed over a time course by chiral HPLC to measure both the conversion of substrate into product and the enantiomeric ratio of the product. This system comprised of the free cells in Tris–HCl buffer at pH 8.0 with 5% sucrose and 1 mg cm^{-3} substrate added in 1,4-dioxane to give 12% v/v of the organic solvent. The results indicated that the enzyme-catalysed reduction proceeds with a high degree of enantioselectivity but that the product was undergoing racemisation, the rate of racemisation being slower than the rate of product formation under these conditions (Table 3).

These studies suggested that reducing the rate of racemisation might allow the isolation of the enantiomerically enhanced product. Since alkaline pH accelerates the rate of product racemisation, the biotransformation was carried out under acidic pH conditions. Over a 4 h reaction at pH 3.0 the product was found to be of > 98% enantiomeric purity. The

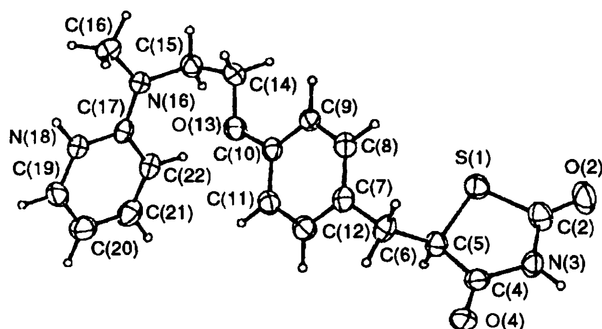


Fig. 1 Compound **6**, *R*-(+) enantiomer of BRL 49653 hydrochloride

ratio of enantiomers of product at pH 3.5 was 95:5 and at pH 4.0 was 91:9 in a similar reaction. The change to acidic pH did not adversely affect the solubility of the substrate. In a 4 h reaction at pH 3.75, with 3 mg cm^{-3} substrate incubated at 28 °C in 12% v/v aqueous 1,4-dioxane, a 93% reduction of compound **1** to product was observed. After extraction of the product, conversion into its dextrorotatory hydrochloride salt gave compound **6** as its monohydrate. X-Ray crystallographic analysis indicated that the stereochemistry at the generated chiral centre was *R* (Fig. 1).*

In summary, a facile biotransformation procedure to produce BRL 49653, and its analogues, utilising *Rhodotorula rubra* CBS 6469 has been found.[15] The procedure is applicable to the synthesis of enantiomerically pure products. Further studies are in progress to examine the applicability of this novel carbon-carbon double bond reduction procedure to substrates other than 5-benzylidenethiazolidine-2,4-diones.

Experimental

General Experimental Details.—M.p.s were recorded on a Büchi 535 capillary melting point apparatus and are uncorrected. Elemental analyses were performed on a Leeman CEC 440 elemental analyser and all values are within ±0.4% of the calculated values. Mass spectroscopy was conducted on a Jeol SX 102 mass spectrometer using electron impact (EI) or fast atom bombardment (FAB) in a 3-nitrobenzyl alcohol–sodium acetate (NOBA–Na) matrix. Compounds characterised by high resolution mass measurement were homogeneous by TLC. ^1H NMR spectra were recorded on a Bruker AM 250, a Jeol GX 270 or a Bruker AMX 400 NMR spectrometer operating at 250.13 MHz, 270.05 MHz and 400.13 MHz, respectively. Spectra were recorded either in $CDCl_3$ or in [$^2H_6$]DMSO solution. Chemical shifts are given in δ (ppm) relative to TMS as the internal standard and coupling constants, *J* are given in Hz. IR spectra were recorded on a Perkin-Elmer 298 infra red spectrophotometer. Optical rotations were measured in path length cells of 10 cm on a Perkin-Elmer Model 241 digital optical polarimeter; $[\alpha]_D^{20}$ values are given in deg cm^2 g^{-1}.

Source of Organisms.—The red yeasts described in this work were obtained from the Centraalbureau Voor Schimmelcultures, Baarn, Netherlands.

Growth Media for Yeasts.—*Medium A.* Yeast extract (10 g) and mycological peptone (20 g) were dissolved in deionised water (1 dm^3) and the pH adjusted to between 7.0–7.2 by the addition of 2 mol dm^{-3} sodium hydroxide solution. After autoclave sterilisation, D-glucose solution (11% v/v of a 30% w/v) was added with filter sterilisation.

Medium B. A mixture of $(NH_4)_2HPO_4$ (13 g), KH_2PO_4 (7 g), yeast extract (3 g), $MgSO_4 \cdot 7H_2O$ (0.8 g), NaCl (0.1 g), $ZnSO_4 \cdot 7H_2O$ (60 mg), $FeSO_4 \cdot 7H_2O$ (90 mg), $CuSO_4 \cdot 5H_2O$ (5 mg) and $MnSO_4 \cdot 4H_2O$ (10 mg) was dissolved in deionised water to a total volume of 900 cm^3 and the pH adjusted to between 7.0–7.2 by the addition of 2 mol dm^{-3} sodium hydroxide solution. After autoclave sterilisation D-glucose (11% v/v of a 40% w/v solution) was added with filter sterilisation.

Screen of Yeasts.—The yeasts were inoculated into flasks of medium B (*vide supra*; 45 cm^3 in a 250 cm^3 Erlenmeyer flask) and incubated for 72 h at 28 °C with shaking. The cells were then separated by centrifugation and resuspended in the original broth volume as a suspension in 0.1 mol dm^{-3} sodium phosphate buffer, pH 8.0 containing sucrose (5% w/v). To the cells from each flask was added the benzylidene substrate dissolved in methyl acetate to give the final concentrations of 250 μg cm^{-3} substrate and 3.6% v/v methyl acetate. Reactions were shaken for 24 h at 28 °C and then assayed by HPLC.

HPLC Conditions.—Reactions were monitored for substrates and products on a Spherisorb ODS reverse phase column (supplied by Phase Separations Ltd.) eluting with 0.05 mol dm^{-3} NaH_2PO_4, pH 7.0–MeCN (60:40) at a flow rate of 2 cm^3 min^{-1} and with UV detection (λ 245 nm). The enantiomers were detected on a Chiral AGP column (supplied by J. T. Baker) eluting with 0.05 mol dm^{-3} NaH_2PO_4, pH 5.5–MeCN (95:5) at a flow rate of 1.2 cm^3 min^{-1} and detecting at λ 242 nm.

General Procedure for the Preparation of 5-(*Benzylidene*)-1,3-*thiazolidine*-2,4-*diones*.— (Z)-5-(4-{2-[*Methyl*(2-*pyridyl*)-*amino*]*ethoxy*}*benzylidene*)*thiazolidine*-2,4-*dione* **1**. 4-{2-[Methyl-(2-pyridyl)amino]ethoxy}benzaldehyde (28.5 g, 0.11 mol) and thiazolidine-2,4-dione (13.0 g, 0.11 mol) were dissolved in toluene (600 cm^3) containing piperidine (0.5 cm^3) and acetic acid (0.5 cm^3). The mixture was heated under reflux for 4 h and then allowed to stand overnight at room temp. The yellow solid was filtered off, washed with diethyl ether and dried under vacuum to give the title benzylidene **1** (24.8 g, 64%), m.p. 196–197 °C (Found: C, 60.9; H, 4.8; N, 11.6. $C_{18}H_{17}N_3O_3S$ requires C, 60.8; H, 4.8; N, 11.8%); ν_{max}(KBr)/cm^{-1} 3380, 1725 and 1690; δ_H(270 MHz; [$^2H_6$]DMSO) 3.07 (3 H, s, NMe), 3.92 (2 H, t, *J* 6.1, NCH_2), 4.22 (2 H, t, *J* 6.1, OCH_2), 6.61 (2 H, m, pyridyl 3-H and 5-H), 7.11 (2 H, d, *J* 8.8, phenyl 3-H and 5-H), 7.52 (3 H, m, phenyl 2-H, 6-H and pyridyl 4-H), 7.73 (1 H, s, olefinic H), 8.09 [1 H, d, *J* 3.6, pyridyl 6-H] and 12.57 (1 H, br, NH; exchanges with D_2O).

By an analogous procedure, the following 5-benzylidenethiazolidine-2,4-diones **4a–f** were prepared:

(Z)-5-(4-{2-[*Benzoxazol*-2-*yl*(*methyl*)*amino*]*ethoxy*}*benzylidenethiazolidine*-2,4-*dione* **4a**. An off-white solid (96%), m.p. 227–229 °C (Found C, 60.6; H, 4.9; N, 10.5. $C_{20}H_{17}N_3O_4S$ requires C, 60.4; H, 4.8; N, 10.6%); ν_{max}(KBr)/cm^{-1} 3300–2800 (br), 1735 and 1690; δ_H(270 MHz; [$^2H_6$]DMSO) 3.23 (3 H, s, NMe), 3.92 (2 H, t, *J* 5.5, NCH_2), 4.34 (2 H, t, *J* 5.5, OCH_2), 6.95–7.40 (6 H, m, phenyl 3-H, 5-H and all benzoxazolyl H), 7.53 (2 H, d, *J* 8.8, phenyl 2-H and 6-H), 7.74 (1 H, s, olefinic H) and 12.5 (1 H, br, NH; exchanges with D_2O).

(Z)-5-(4-{4-[*Methyl*(2-*pyridyl*)*amino*]*butoxy*}*benzylidene*)-*thiazolidine*-2,4-*dione* **4b**. A pale yellow solid (85%), m.p. 157–159 °C; ν_{max}(KBr)/cm^{-1} 3600–3250br, 1730, 1685 and 1600; δ_H(270 MHz; [$^2H_6$]DMSO) 1.70 (4 H, m, $NCH_2CH_2CH_2CH_2O$), 2.98 (3 H, s, NMe), 3.56 (2 H, t, *J* 6.9, NCH_2), 4.07 (2 H, t, *J* 6.9, CH_2O), 6.51 (1 H, m, pyridyl 5-H), 6.59 (1 H, d, *J*

* The *S*-(−)-enantiomer of BRL 49653 may be prepared by the resolution of BRL 49653 with (−)-quinine in ethyl acetate. After conversion to its hydrochloride salt, the properties of *S*-(−)-BRL 49653 were: m.p. 122–3 °C, $[\alpha]_D^{20}$ −111.9 (*c* 0.5, MeOH), enantiomer ratio 98.9:1.1 by chiral HPLC.[16]

8.8, pyridyl 3-H), 7.07 (2 H, d, *J* 8.8, phenyl 3-H and 5-H), 7.45 (1 H, m, pyridyl 4-H), 7.52 (2 H, d, *J* 8.8, phenyl 2-H and 6-H), 7.74 (1 H, s, olefinic H), 8.06 (1 H, m, pyridyl 6-H) and 8.50 (1 H, br, NH; exchanges with D_2O).

(Z)-5-(4-{2-[*Methyl(2-quinolyl)amino]ethoxy}benzylidene)-thiazolidine*-2,4-*dione* **4c**. A yellow solid (55%), m.p. 186–187 °C (Found: C, 64.8; H, 4.6; N, 10.3; S, 7.8. $C_{22}H_{19}N_3O_3S$ requires C, 65.1; H, 4.7; N, 10.4; S, 7.9%); ν_{max}(KBr)/cm^{-1} 3250–2700, 1730 and 1680; δ_H(270 MHz; [$^2H_6$]DMSO) 3.23 (3 H, s, NMe), 4.06 (2 H, t, *J* 5.9, NCH_2), 4.32 (2 H, t, *J* 5.9, OCH_2), 7.10–7.30 (4 H, m, phenyl 3-H and 5-H and quinolyl 3-H and 6-H), 7.45–7.60 (4 H, m, phenyl 2-H and 6-H) and quinolyl 7-H and 8-H), 7.68 (1 H, d, *J* 7.7, quinolyl 5-H), 7.74 (1 H, s, olefinic H), 8.03 (1 H, d, *J* 9.4, quinolyl 4-H) and 12.50 (1 H, br, NH; exchanges with D_2O).

(Z)-5-(4-{2-[1-*Isoquinolyl(methyl)amino]ethoxy}benzylidene)thiazolidine*-2,4-*dione* **4d**. A yellow solid (77%) [Found: MH^+ (FAB, NOBA–Na) 406. $C_{22}H_{19}N_3O_3S$ requires *M* 406]; δ_H(270 MHz; [$^2H_6$]DMSO) 3.11 (3 H, s, NMe), 3.79 (2 H, t, *J* 5.8, NCH_2), 4.40 (2 H, t, *J* 5.8, OCH_2), 7.09 (2 H, d, *J* 8.8, phenyl 3-H and 5-H), 7.31 (1 H, d, *J* 5.8, isoquinolyl 4-H), 7.53 [3 H, m, phenyl 2-H and 6-H and isoquinolyl 7-H), 7.68 (1 H, m, isoquinolyl 6-H), 7.74 (1 H, s, olefinic H), 7.84 (1 H, d, *J* 7.7, isoquinolyl 5-H), 8.06 (1 H, d, *J* 5.8, isoquinolyl 3-H), 8.23 (1 H, d, *J* 8.3, isoquinolyl 8-H) and 12.0 (1 H, br, NH; exchanges with D_2O).

(Z)-5-(4-{2-[3-*Chloro-2-pyridyl(methyl)amino]ethoxy}-benzylidene)thiazolidine*-2,4-*dione* **4e**. A yellow solid (70%). δ_H(250 MHz; [$^2H_6$]DMSO) 3.02 (3 H, s, NMe), 3.76 (2 H, t, *J* 5.8, NCH_2), 4.30 (2 H, t, *J* 5.8, OCH_2), 6.91 (1 H, dd, *J* 7.7 and 4.7, pyridyl 5-H), 7.03 (2 H, d, *J* 8.8, phenyl 3-H and 5-H), 7.52 (2 H, d, *J* 8.8, phenyl 2-H and 6-H), 7.73 (1 H, s, olefinic H), 7.76 (1 H, dd, *J* 7.7 and 1.6, pyridyl 4-H) and 8.16 (1 H, dd, *J* 4.7 and 1.6, pyridyl 6-H) and 12.50 (1 H, br, NH; exchanges with D_2O).

(Z)-5-(4-{2-[3,5-*Dichloro-2-pyridyl(methyl)amino]ethoxy}-benzylidene)thiazolidine*-2,4-*dione* **4f**. A yellow solid (72%), m.p. 176–177 °C (Found: C, 50.75; H, 3.6; N, 9.9. $C_{18}H_{15}Cl_2N_3O_3S$ requires C, 50.95; H, 3.6; N, 9.9%); ν_{max}(KBr)/cm^{-1} 3200–2500 br, 1730 and 1685; δ_H(270 MHz; [$^2H_6$]DMSO) 3.11 (3 H, s, NMe), 3.86 (2 H, t, *J* 5.8, NCH_2), 4.37 (2 H, t, *J* 5.8, OCH_2), 7.09 (2 H, d, *J* 8.8, phenyl 3-H and 5-H), 7.59 (2 H, d, *J* 8.8, phenyl 2-H and 6-H), 7.81 (1 H, s, olefinic H), 8.01 (1 H, d, *J* 2.2, pyridyl 4-H), 8.27 (1 H, d, *J* 2.2, pyridyl 6-H) and 12.56 (1 H, br, NH; exchanges with D_2O).

General Procedure for Biocatalytic Reductions Using Rhodotorula rubra *CBS* 6469 *to Give Racemic Product* (*Method A*).—5-(4-{2-[*Methyl(2-pyridyl)amino]ethoxy}benzyl)thiazolidine*-2,4-*dione* **2**. A loopful of *Rhodotorula rubra* CBS 6469 was used to inoculate a flask of medium A (90 cm^3 in a 500 cm^3 Erlenmeyer flask) and this was incubated at 28 °C for 72 h with continuous shaking, after which 1 cm^3 of the broth was taken and used to inoculate a similar flask, which was incubated for 48 h prior to centrifugation. The cells were then resuspended in 0.1 mol dm^{-3} Tris–HCl buffer (pH 8.0) containing 5% w/v sucrose (69 cm^3). To 40 cm^3 of this cell suspension in a 250 cm^3 flask was added the benzylidene compound **1** (7.5 cm^3 of a 5 mg cm^{-3} solution in 1,4-dioxane) and the mixture shaken at 28 °C for 22 h. After removal of the 1,4-dioxane by evaporation under reduced pressure and the addition of water (50 cm^3), the mixture was continuously extracted with dichloromethane for 18 h and the extract dried ($MgSO_4$), filtered and evaporated. The residue was chromatographed on silica gel using methanol–dichloromethane (2:98) as the eluent to give the reduced product **2** (15 mg, 42%), m.p. 154–155 °C (from MeOH). Reduction of the benzylidene compound **1** by the dissolving magnesium metal procedure (Method C, below) also afforded compound **2** (84%), m.p. 153–155 °C, identical with the biocatalytically reduced material. (Found C, 60.2; H, 5.3; N, 11.7. $C_{18}H_{19}N_3O_3S$ requires C, 60.5; H, 5.4; N, 11.8%); ν_{max}(KBr)/cm^{-1} 3400br, 1735 and 1695; δ_H(270 MHz; [$^2H_6$]DMSO) 3.05 (1 H, dd, *J* 14.3 and 9.1, ArC*H*HCH), 3.06 (3 H, s, NMe), 3.29 (1 H, dd, *J* 14.3 and 4.4, ArCH*H*CH), 3.89 (2 H, t, *J* 5.5, NCH_2), 4.10 (2 H, t, *J* 5.5, OCH_2), 4.85 (1 H, dd, *J* 9.1 and 4.4, $ArCH_2CH$), 6.55 (1 H, m, pyridyl 5-H), 6.63 (1 H, d, *J* 8.8, pyridyl 3-H), 6.87 (2 H, d, *J* 8.8, phenyl 3-H and 5-H), 7.13 (2 H, d, *J* 8.8, phenyl 2-H and 6-H), 7.50 (1 H, m, pyridyl 4-H), 8.07 (1 H, dm, ^3J 4.8, pyridyl 6-H) and 12.0 (1 H, br, NH; exchanges with D_2O). The above enzymatic procedure has been conducted on a scale of up to 3 g of substrate.

General Procedure for Catalytic Hydrogenation (*Method B*).—5-(4-{2-[*Benzoxazol-2-yl(methyl)amino]ethoxy}benzyl)-thiazolidine*-2,4-*dione* **5a**. A solution of the benzylidene compound **4a** (1.5 g, 3.8 mmol) in dry 1,4-dioxane (80 cm^3) was hydrogenated in the presence of 10% palladium on charcoal (2.0 g) at 18 °C and at atmospheric pressure until the hydrogen uptake had ceased. The solution was filtered through Celite, the filter pad was washed with dry 1,4-dioxane (100 cm^3) and the combined filtrates were evaporated to dryness under reduced pressure. The resulting oil was chromatographed on silica gel using methanol–dichloromethane (2:98) as the eluent to afford the reduced compound **5a** (0.93 g, 61%) as a colourless solid, m.p. 147–149 °C (from MeOH). Reduction of compound **4a** by the biocatalytic procedure (Method A) also afforded compound **5a** (49%), identical with the chemically prepared material. (Found: C, 60.65; H, 4.3; N, 10.6. $C_{20}H_{19}N_3O_4S$ requires C, 60.7; H, 4.3; N, 10.6%); ν_{max}(KBr)/cm^{-1} 3400, 1740 and 1690; δ_H(270 MHz; [$^2H_6$]DMSO) 3.04 (1 H, dd, *J* 14 and 9.1, ArC*H*HCH), 3.22 (3 H, s, NMe), 3.28 (1 H, dd, *J* 14 and 4.4, ArCH*H*CH), 3.84 (2 H, t, *J* 5.2, NCH_2), 4.23 (2 H, t, *J* 5.2, OCH_2), 4.85 (1 H, dd, *J* 9.1 and 4.4, $ArCH_2CH$), 6.80–7.40 (8 H, m, ArH) and 12.00 (1 H, br, NH; exchanges with D_2O).

General Procedure for Dissolving Magnesium Metal Reductions (*Method C*).—5-(4-{4-[*Methyl(2-pyridyl)amino]butoxy}-benzylthiazolidine*-2,4-*dione* **5b**. A mixture of the benzylidene compound **4b** (3.00 g, 7.8 mmol), magnesium turnings (1.00 g, 41.6 mmol), iodine (5 mg) and methanol (100 cm^3) was warmed gently until the evolution of hydrogen commenced. A further portion of magnesium (6.50 g, 0.27 mol) was added over a period of 5 min and the mixture then cooled in an ice bath and stirred for a further 2 h. The ice bath was then removed, an additional portion of methanol (25 cm^3) was added to facilitate stirring, which was continued for a further 16 h. The mixture was diluted with hydrochloric acid (2 mol dm^{-3}; 400 cm^3) and stirred with the addition of conc. HCl as required to maintain pH 1 until all the solid had dissolved. The mixture was adjusted to pH 7.5 by the addition of conc. aq. ammonia solution and extracted with dichloromethane (3 × 500 cm^3). The combined dichloromethane layers were washed with water (2 × 1 dm^3) and brine (1 dm^3), dried ($MgSO_4$) and evaporated. The residue was chromatographed on silica gel using methanol–dichloromethane (1.5:98.5) as the eluent to afford compound **5b** (0.97 g, 32%) as a gum. Crystallisation from dichloromethane–hexane afforded an analytical sample, m.p. 108–111 °C. Reduction of compound **4b** by the biocatalytic procedure (Method A) also afforded compound **5b** (38%), identical with the chemically prepared material. (Found: C, 62.2; H, 6.25; N, 10.9. $C_{20}H_{23}N_3O_3S$ requires C, 62.3; H, 6.0; N, 10.9%); ν_{max}(KBr)/cm^{-1} 3400br and 1695; δ_H(270 MHz; $CDCl_3$) 1.76 (4 H, m, $NCH_2CH_2CH_2$-CH_2O), 3.03 (3 H, s, NMe), 3.11 (1 H, dd, *J* 14.0 and 9.3, ArC*H*HCH), 3.43 (1 H, dd, *J* 14.0 and 3.9, ArCH*H*CH), 3.59 (2 H, m, NCH_2), 3.97 (2 H, m, OCH_2), 4.25 (1 H, br, NH;

exchanges with D_2O), 4.49 (1 H, dd, *J* 9.3 and 3.9, $ArCH_2CH$), 6.50 (2 H, m, pyridyl 3-H and 5-H), 6.83 (2 H, d, *J* 8.8, phenyl 3-H and 5-H), 7.13 (2 H, d, *J* 8.8, phenyl 2-H and 6-H), 7.45 (1 H, m, pyridyl 4-H) and 8.13 (1 H, m, pyridyl 6-H).

5-(4-{2-[*Methyl(2-quinolyl)amino*]*ethoxy*}*benzyl)thiazolidine*-2,4-*dione* **5c**. Reduction of compound **4c** by Method A afforded compound **5c** (32%), as a glassy solid [Found: M^+ (EI) 407.128. $C_{22}H_{21}N_3O_3S$ requires *M* 407.130]; δ_H (250 MHz; [$^2H_6$]DMSO) 3.05 (1 H, dd, *J* 14.1 and 9.0, Ar*CH*HCH), 3.22 (3 H, s, NMe), 3.27 (1 H, dd, *J* 14.1 and 4.2, ArCH*H*CH), 4.02 (2 H, t, *J* 5.6, NCH_2), 4.20 (2 H, t, *J* 5.6, OCH_2), 4.86 (1 H, dd, *J* 9.0 and 4.2, $ArCH_2CH$), 6.93 (2 H, d, *J* 8.7, phenyl 3-H and 5-H), 7.10–7.25 (4 H, m, phenyl 2-H and 6-H and quinolyl 3-H and 6-H), 7.47–7.59 (2 H, m, quinolyl 7-H and 8-H, 7.67 (1 H, d, *J* 7.7, quinolyl 5-H), 8.02 (1 H, d, *J* 9.2, quinolyl 4-H) and 12.0 (1 H, br, NH; exchanges with D_2O).

5-(4-{2-[1-*Isoquinolyl(methyl)amino*]*ethoxy*}*benzyl)thiazolidine*-2,4-*dione* **5d**. Reduction of compound **4d** by Method A afforded compound **5d** (59%), m.p. 139–141 °C (from MeOH) [Found: M^+ (EI), 407.134. $C_{22}H_{21}N_3O_3S$ requires *M*, 407.1382]; ν_{max}(KBr)/cm^{-1} 3400br, 1751 and 1701; δ_H(250 MHz; [$^2H_6$]DMSO) 3.04 (1 H, dd, *J* 14.2 and 9.0, Ar*CH*HCH), 3.10 (3 H, s, Me), 3.30 (1 H, dd, *J* 14.2 and 4.3, ArCH*H*CH), 3.74 (2 H, t, *J* 5.7, NCH_2), 4.30 (2 H, t, *J* 5.7, OCH_2), 4.86 (1 H, dd, *J* 9.0 and 4.3, $ArCH_2CH$), 6.87 (2 H, d, *J* 8.6, phenyl 3-H and 5-H), 7.14 (2 H, d, *J* 8.6, phenyl 2-H and 6-H), 7.31 (1 H, d, *J* 5.7, isoquinolyl 4-H), 7.54 (1 H, m, isoquinolyl 7-H), 7.67 (1 H, m, isoquinolyl 6-H), 7.85 (1 H, d, *J* 8.0, isoquinolyl 5-H), 8.05 (1 H, d, *J* 5.7, isoquinolyl 3-H), 8.25 (1 H, d, *J* 8.3, isoquinolyl 8-H) and 12.50 (1 H, br, NH; exchanges with D_2O).

5-(4-{2-[3-*Chloro*-2-*pyridyl(methyl)amino*]*ethoxy*}*benzylthiazolidine*-2,4-*dione* **5e**. Reduction of compound **4e** by Method B afforded compound **5e** (7%) together with some dechlorinated material **2**. Reduction of compound **4e** by Method A gave only the desired compound **5e** (57%), m.p. 118–119 °C (from MeOH) [Found C, 54.9; H, 4.5; N, 10.6; M^+ (EI), 391.0757. $C_{18}H_{18}ClN_3O_3S$ requires C, 55.2; H, 4.6; N, 10.7%; *M* 391.0758]; ν_{max}(KBr)/cm^{-1} 3400 (br), 1745 and 1685; δ_H(270 MHz; [$^2H_6$]DMSO) 3.02 (3 H, s, NMe), 3.05 (1 H, dd, *J* 14.3 and 9.1, Ar*CH*HCH), 3.27 (1 H, dd, *J* 14.3 and 4.4, ArCH*H*CH), 3.73 (2 H, t, *J* 5.7, NCH_2), 4.19 (2 H, t, *J* 5.7, OCH_2), 4.84 (1 H, dd, *J* 9.1 and 4.4, $ArCH_2CH$), 6.80 (2 H, d, *J* 8.6, phenyl 3-H and 5-H), 6.90 (1 H, dd, *J* 7.7 and 4.7, pyridyl 5-H), 7.12 (2 H, d, *J* 8.6, phenyl 2-H and 6-H), 7.72 (1 H, dd, *J* 7.7 and 1.6, pyridyl 4-H), 8.14 (1 H, dd, *J* 4.6 and 1.6, pyridyl 6-H) and 12.00 (1 H, br, NH; exchanges with D_2O).

5-(4-{2-[3,5-*Dichloro*-2-*pyridyl(methyl)amino*]*ethoxy*}*benzyl)thiazolidine*-2,4-*dione* **5f**.—Reduction of compound **4f** by Method A gave compound **5f** (51%). δ_H(250 MHz; [$^2H_6$]DMSO) 3.02 (3 H, s, NMe), 3.03 (1 H, dd, *J* 14.0 and 8.9, Ar*CH*HCH), 3.29 (1 H, dd, *J* 14.0 and 4.3, ArCH*H*CH), 3.74 (2 H, t, *J* 5.7, NCH_2), 4.18 (2 H, t, *J* 5.7, OCH_2), 4.86 (1 H, dd, *J* 8.9 and 4.3, $ArCH_2CH$), 6.79 (2 H, d, *J* 8.3, phenyl 3-H and 5-H), 7.12 (2 H, d, *J* 8.3, phenyl 2-H and 6-H), 7.95 (1 H, d, *J* 1.6, pyridyl 4-H), 8.20 (1 H, d, *J* 1.6, pyridyl 6-H) and 12.00 (1 H, br, NH; exchanges with D_2O).

Reduction of (Z)-5-(4-{2-[*Methyl(2-pyridyl)amino*]*ethoxy*}-*benzylidene)thiazolidine*-2,4-*dione* **1** *by Immobilised* Rhodotorula rubra *CBS* 6469 *to give* (±)-5-(4-{2-[*Methyl(2-pyridyl)amino*]*ethoxy*}*benzyl)thiazolidine*-2,4-*dione* **2**.—The yeast cells were grown as before and after centrifugation, the cells from 90 cm^3 of the broth were resuspended in Tris–HCl buffer pH 8.0 (12.5 cm^3) containing 5% sucrose w/v. An equal volume of 2% w/v sodium alginate solution, in the same buffer, also containing 5% sucrose w/v was added and the cells immobilised into beads by standard methodology.[17] The beads were washed again in buffer containing 5% sucrose w/v and identical buffer solution added to give a total volume of 40 cm^3 in a 250 cm^3 flask, to which was added compound **1** (7.5 cm^3 of a 5 mg cm^{-3} solution in 1,4-dioxane). The suspension was shaken at 28 °C for 22 h after which HPLC analysis indicated an 87% conversion. The supernatant was decanted and the beads washed with 50 cm^3 of 20% v/v 1,4-dioxane in buffer. The aqueous solution was extracted with dichloromethane, which was then dried ($MgSO_4$) and evaporated to give compound **2** (51%), identical by ^1H NMR spectroscopy to authentic material.[8]

Reduction of (Z)-5-(4-{2-[*Methyl(2-pyridyl)amino*]*ethoxy*}*benzylidene)thiazolidine*-2,4-*dione* **1** *by* Rhodotorula rubra *CBS* 6469 *to give* (R)-(+)-5-(4-{2-[*Methyl(2-pyridyl)amino*]-*ethoxy*}*benzyl)thiazolidine*-2,4-*dione* **6**.—The red yeast was grown as before and the centrifuged cell pellet from 220 cm^3 of growth medium was resuspended in 88 cm^3 of 0.1 mol dm^{-3} citrate buffer pH 3.75, containing 5% w/v sucrose. To this was added 12 cm^3 of an 8.33 mg cm^{-3} solution of compound **1** in 1,4-dioxane. After shaking at 28 °C for 3 h 20 min the cells were removed and washed in buffer–1,4-dioxane. Chiral HPLC analysis indicated an enantiomeric ratio of 94:6. The solution was reduced to 2/3 of its original volume under reduced pressure without heating and then basified to pH 8 (10% aq. ammonia) and rapidly extracted with dichloromethane (3 × 50 cm^3). The combined organic extracts were dried ($MgSO_4$), filtered, evaporated to dryness under reduced pressure (< 25 °C) and the gummy residue dissolved in water (10 cm^3) containing concentrated HCl (0.2 cm^3). After cooling to 2 °C for 24 h, the solid was filtered off and dried to give compound **6** as its hydrochloride monohydrate (18%), m.p. 123–124 °C. (Found: C, 52.6; H, 5.45; Cl, 8.65; N, 10.2; S, 7.7. $C_{18}H_{19}N_3O_3S{\cdot}HCl{\cdot}H_2O$ requires C, 52.5; H, 5.4; Cl, 8.6; N, 10.2; S, 7.8%); $[\alpha]_D^{20}$ +107.8 (*c* 0.5, MeOH); enantiomeric purity >99.5% by chiral HPLC; ν_{max}(KBr)/cm^{-1} 3360, 3200–2500 (br), 1745 and 1700; δ_H(400 MHz; [$^2H_6$]DMSO) 3.05 (1 H, dd, *J* 14.1 and 8.9, Ar*CH*HCH), 3.28 (1 H, dd, *J* 14.1 and 4.5, ArCH*H*CH), 3.29 (3 H, s, NMe), 3.51, (2 H, br, H_2O; exchanges with D_2O), 4.08 (2 H, t, *J* 5.2, NCH_2), 4.22 (2 H, t, *J* 5.2, OCH_2), 4.85 (1 H, dd, *J* 8.9 and 4.5, $ArCH_2CH$), 6.82 (2 H, d, *J* 8.7, phenyl 3-H and 5-H), 6.93 (1 H, t, *J* 6.6, pyridyl 5-H), 7.14 (2 H, d, *J* 8.7, phenyl 2-H and 6-H), 7.31 (1 H, d, *J* 9.2, pyridyl 3-H), 7.98 (2 H, m, pyridyl 4-H and 6-H), 12.00 (1 H, s, NH; exchanges with D_2O), 14.01 (1 H, br, HCl; exchanges with D_2O).

X-Ray Crystal Data for (R)-(+)-5-(4-{2-[*Methyl(2-pyridyl)amino*]*ethoxy*}*benzyl)thiazolidine*-2,4-*dione* **6**.—$C_{18}H_{19}N_3O_3S{\cdot}HCl{\cdot}H_2O$, $M = 411.9$. Cell dimensions (at 223K): $a = 4.538(2)$, $b = 11.816(3)$, $c = 18.567(4)$ Å, $\beta = 90.60(2)°$, $V = 995.5(6)$ Å^3, space group $P2_1$, $Z = 2$, $D_c = 1.374$ g cm^{-3} for colourless needles of dimensions 0.70 × 0.20 × 0.02 mm. Intensity data were collected on an Enraf–Nonius CAD4 diffractometer in $\omega/2\theta$ scan mode using Mo-K_α radiation ($\lambda = 0.71073$ Å) and were corrected for absorption; 4118 reflections were measured ($2° \leqslant 2\theta \leqslant 50°$, $-4 \leqslant h \leqslant 2$, $0 \leqslant k \leqslant 13$, $-18 \leqslant l \leqslant 18$), 2716 unique including Friedel mates. The structure was solved by use of the MULTAN80 program.* Atomic positions for non-hydrogen atoms were eventually refined with ansiotropic

* P. Main, S. J. Fiske, S. E. Hull, L. Lessinger, G. Germain, J. P. Declercq and M. M. Woolfson, MULTAN80. A system of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data. Univs. of York, England and Louvain, Belgium.

displacement parameters. Hydrogen atoms attached to carbons were held fixed at geometrically calculated positions in the final refinement with isotropic temperature factors assigned as 1.3 (B_{eq}) of the attached atom. Hydrogens attached to heteroatoms were located from difference Fourier maps and were refined. The full-matrix least-squares refinement (on F) converged (max $\Delta/\sigma = 0.00$) to values of the conventional crystallographic residuals $R = 0.030$, $R_w = 0.038$ for observed data. The function minimised was $\Sigma w(|F_o| - |F_c|)^2$. Weights w, were assigned to the data as $w = 1/\sigma^2(F_o) = [\sigma^2(I_c) + (0.051I)^2]$. A final difference Fourier map was featureless with a maximum residual density between ± 0.297 eÅ^{-3}. To assign configuration, coordinates of the model were inverted and re-refinement proceeded to give a weighted crystallographic residual (R_w) of 0.0400. The R-factor ratio of 1.052 is statistically significant at the 99.95% level based on a refinement of 255 variables with 2342 observations. Friedel mates calculated to be most directly effected by anomalous dispersion were remeasured using copper radiation to take advantage of the stronger anomalous signal. These data also confirmed the original (R)-configuration assignment as 100% of the measured pairs gave agreement in sign and magnitude. Atomic coordinates, bond lengths and angles have been deposited with the Cambridge Crystallographic Data Centre.*

* For details see Instructions for Authors (1994), *J. Chem. Soc., Perkin Trans. 1*, 1994, Issue 1.

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