Chemoenzymatic Synthesis and Fungicidal Activity of the Four Pure Stereoisomers of a New Morpholine Derivative†

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Four optically pure stereoisomers of 2,6-dimethyl-4-[2-methyl-3-[3-(cyclopropylmethoxy)phenyl]propyl]-morpholine (1), a new broad-spectrum morpholine fungicide, were prepared starting from the chiral precursor 2-methyl-3-[3-(benzyloxy)phenyl]-1-propanol (2). Optically active 2 was obtained by lipase-catalyzed kinetic resolution of (*R,S*)-2 and by stereoselective bakers' yeast reduction of 2-methyl-3-[3-(benzoyloxy)phenyl]propenal (4). The biological activity of the pure stereoisomers has been evaluated in vitro against a variety of fungi and in vivo against *Erysiphe graminis* on wheat and *Helminthosporium teres* on barley.

INTRODUCTION

2,6-Dimethyl-4-[2-methyl-3-[3-(cyclopropylmethoxy)-phenyl]propyl]morpholine (1) (Figure 1) is a new morpholine fungicide active against a number of *Ascomycetes* and *Deuteromycetes*. It exhibits a good selective control of *Helminthosporium teres* as compared to the commercial morpholine compound fenpropimorph. Four stereoisomers are possible for compound 1, because it contains a chiral center on the propyl chain and the 2,6-dimethylmorpholine moiety exists in the cis and trans configurations. Each stereoisomer is expected to show a different set of interactions with macromolecular structures such as serum proteins, antibodies, receptors, and metabolizing enzymes, which results in different biological response (Pommer, 1985).

At the present time, there is growing scientific interest in the biological behavior of chiral compounds to provide crop protection agents with better performance, higher selectivity, and toxicological and environmental advantages (Fuchs, 1988; Tombo and Bellus, 1991; Bianchi et al., 1991). Consequently, we had an interest in preparing the four pure stereoisomers of fungicide 1 to compare their biological activity.

Techniques for stereospecific synthesis and separation of isomers, including enantiomers, are rapidly evolving. Synthesis based on biotransformations can offer significant advantages over classical chemical methods involving resolution of racemates, chiral pool templates, and asymmetric synthetic reagents, especially in the preparation of physiologically active compounds. Advantages of using purified enzymes and microorganisms in biotransformations include the possibility of carrying out a wide range of organic reactions under mild temperature and pH conditions, with high tolerance for variations of substrate structure. Recently, several examples of regioselective and enantioselective transformations brought off by hydrolases, oxidoreductases, and ligases have been reported (Jones, 1986; Klibanov, 1990; Boland et al., 1991). Hydrolytic enzymes, such as lipases or proteases, have been proposed for kinetic resolution of alcohols, both in hydrolysis (Francalanci et al., 1987; Laumen and Schneider, 1988) and in transesterification reactions (Wang et al., 1988; Bianchi et al., 1988). *Saccharomyces cerevisiae* (bakers' yeast) has been employed for asymmetric reductive biotransformation of a variety of compounds containing a carbonyl group or a carbon–carbon double bond (Servi, 1990).

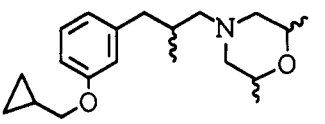

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Figure 1. (±)-*cis/trans*-1.

In this paper we describe the preparation of the optically pure intermediate 2, by lipase-catalyzed stereoselective transesterification of racemic alcohol 2 and by bakers'-yeast-catalyzed reduction of the aldehyde 4, and its use in the synthesis of the four stereoisomers of the fungicide 1. Moreover, the availability of the four stereoisomers of 1 prompted us to perform in vitro fungicidal tests on a number of *Deuteromycetes*, as well as greenhouse tests on *Erysiphe graminis* on wheat and *H. teres* on barley.

EXPERIMENTAL PROCEDURES

Apparatus. Optical rotation was measured with a Perkin-Elmer 241 polarimeter.

^{1}H NMR spectra were recorded in $CDCl_3$ solution [$(CH_3)_4Si$ as internal standard] on a Bruker AM 300 instrument.

GLC analyses were carried out on a Carlo Erba HRGC 5300, equipped with a 2 m × 4 mm column, at 100–280 °C and with a flame ionization detector.

The optical purity of 2 was determined with a Waters HPLC system (two Model 501 pumps, Model U6K injector, Model 484 variable-wavelength UV detector, NEC APC IV personal computer) equipped with a Daicel Chiralcel OD chiral column [eluant, hexane/2-propanol 9:1 (v/v); flow, 0.8 mL/min].

Silica gel column chromatography was conducted on silica gel 60, 230–400-mesh ATSM (Merck).

Reagents. Lipase Amano PS from *Pseudomonas cepacia* (30 units/mg) was purchased from Amano Chemical Co. Porcine pancreatic lipase (13 units/mg) was purchased from Sigma Chemical Co. Bakers' yeast (*S. cerevisiae*) was Distillerie Italiane brand purchased from Eridania.

All of the organic chemicals used were purchased from Fluka Chemie.

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Procedures. (±)-*2-Methyl-3-[3-(benzyloxy)phenyl]propyl Acetate* (3). (±)-2-Methyl-3-[3-(benzyloxy)phenyl]-1-propanol (2) (5 g, 19 mmol) was added to acetic anhydride (80 mL) and refluxed for 10 h. The excess anhydride was removed in vacuo, and the residue was dissolved in ethyl acetate, washed with ether, dried (Na_2SO_4), and evaporated to dryness. Chromatography on silica gel using hexane/ether 9:1 as eluant afforded 5.2 g of (±)-3: ^1H NMR ($CDCl_3$) δ 7.5–6.8 (9 H, m), 5.1 (2 H, s), 3.8 (2 H, m), 2.8–2.3 (2 H, m), 2.1 (1 H, m), 2.0 (3 H, s), 0.9 (3 H, d). Anal. Calcd for $C_{19}H_{22}O_3$: C, 76.51; H, 7.38. Found: C, 76.45; H, 7.35.

Enzymatic Hydrolysis of (±)-*2-Methyl-3-[3-(benzyloxy)phenyl]propyl Acetate* (3). To a magnetically stirred mixture of (±)-3 (1.5 g, 5 mmol) and phosphate buffer, 0.01 N, pH 7 (40 mL) at 30 °C, was added lipase Amano PS (100 mg), and the pH was kept at 7 with 1 N aqueous sodium hydroxide by using a pH-stat.

The hydrolysis was stopped at 45% conversion (14 h), then the reaction mixture was extracted with the ethyl acetate, and the organic layer was dried (Na_2SO_4) and evaporated to dryness. Chromatography on silica gel using hexane/ether 95:5 as eluant afforded 750 mg of (*R*)-(−)-3, ee = 22% and $[\alpha]^{25}_D$ −2.5° (*c* 1, $CHCl_3$), and 520 mg of (*S*)-(−)-2-methyl-3-[3-(benzyloxy)phenyl]-1-propanol (2), ee = 30% and $[\alpha]^{25}_D$ −3.1° (*c* 1, $CHCl_3$): ^1H NMR ($CDCl_3$) δ 7.5–6.8 (9 H, m), 5.1 (2 H, s), 3.45 (2 H, m), 2.8–2.3 (2 H, m), 2.0–1.8 (1 H, m), 0.9 (3 H, d). Anal. Calcd for $C_{17}H_{20}O_2$: C, 79.70; H, 7.81. Found: C, 79.60; H, 7.80.

Enzymatic Transesterification of (±)-*2-Methyl-3-[3-(benzyloxy)phenyl]-1-propanol* (2). Lipase Amano PS (500 mg) was added to a solution of (±)-2 (8 g, 31 mmol) in anhydrous ethyl acetate (150 mL), and the suspension was shaken at 25 °C. The reaction was stopped at 55% conversion (4 h) by filtering off the enzyme. The solution was evaporated to dryness, and the products were purified by chromatography on silica gel using hexane/ether 95:5 as eluant, affording 3 g of (*R*)-(+)-2, ee = 95% and $[\alpha]^{25}_D$ +10.1° (*c* 1, $CHCl_3$), and 4.8 g of (*S*)-(+)-3, $[\alpha]^{25}_D$ +9.6° (*c* 1, $CHCl_3$). The same reaction, stopped at 45% conversion, afforded 4 g of (*R*)-(+)-2, ee = 79%, $[\alpha]^{25}_D$ +8.4 (*c* 1, $CHCl_3$), and 3.9 g of (*S*)-(+)-3, $[\alpha]^{25}_D$ +11.3° (*c* 1, $CHCl_3$).

Alkaline Hydrolysis of (*S*)-(+)-*2-Methyl-3-[3-(benzyloxy)phenyl]propyl Acetate* (3). (*S*)-(+)-3, $[\alpha]^{25}_D$ +11.3° (*c* 1, $CHCl_3$) (3.9 g, 13 mmol), was dissolved in a 1 N solution of sodium hydroxide in absolute ethanol (40 mL). The solution was stirred for 2 h at 25 °C and then evaporated under vacuum. The residue was extracted with dichloromethane, and the organic layer was washed with water, dried (Na_2SO_4), and evaporated to dryness. Chromatography on silica gel using hexane/ether 95:5 as eluant afforded 3.0 g of (*S*)-(−)-2, ee > 98% and $[\alpha]^{25}_D$ −10.5° (*c* 1, $CHCl_3$).

Bakers' Yeast Reduction of 2-Methyl-3-[3-(benzyloxy)phenyl]propenal (4). A solution of 2-methyl-3-[3-(benzyloxy)phenyl]-propenal (4) (9 g, 34 mmol) in ethanol (15 mL) was added (1.5 h) to a stirred suspension of bakers' yeast (562 g) in a distilled water solution (1.7 L) of D-glucose (168 g) at 28 °C. The mixture was stirred at 220 rpm for 24 h and then filtered through Celite, and the filtrate was extracted with ethyl ether. The extract was dried (Na_2SO_4) and evaporated in vacuo. Chromatography on silica gel using hexane/ethyl acetate 9:1 as eluant afforded 1.5 g of unreacted 4 and 5.0 g of (*S*)-(−)-2, ee = 94% and $[\alpha]^{25}_D$ −10.0° (*c* 1, $CHCl_3$).

(*R*)-(−)-*3-(2-Methyl-3-iodopropyl)phenol* (5). A mixture of (*R*)-(+)-2, ee = 95% (11.4 g, 43 mmol), trimethylchlorosilane (9.8 g, 91 mmol), and sodium iodide (13.7 g, 90 mmol) in anhydrous acetonitrile (300 mL) was refluxed under nitrogen. After 48 h, sodium iodide (6.9 g) and trimethylchlorosilane (4.9 g) were added, and the reaction mixture was refluxed for a further 48 h. The salts were filtered off and the solvent was evaporated under vacuum. Chromatography on silica gel using hexane/ethyl acetate 9:1 as eluant afforded 7.0 g of (*R*)-(−)-3-(2-methyl-3-iodopropyl)-phenol (5), $[\alpha]^{25}_D$ −33.0° (*c* 1, $CHCl_3$): ^1H NMR ($CDCl_3$) δ 7.4–6.6 (4 H, m), 5.3 (1 H, s), 3.3–3.0 (2 H, m), 2.7–2.4 (2 H, m), 1.7 (1 H, m), 1.0 (3 H, d). Anal. Calcd for $C_{10}H_{13}IO$: C, 43.49; H, 4.71. Found: C, 43.40; H, 4.65.

(*S*)-(+)-*3-(2-Methyl-3-iodopropyl)phenol* (5). The above-described procedure, starting from (*S*)-(−)-2, ee >98% (8 g, 30 mmol), afforded 5.5 g of (*S*)-(+)-5, $[\alpha]^{25}_D$ +34.7° (*c* 1, $CHCl_3$).

trans- and cis-(R)-(+)-2,6-Dimethyl-4-[2-methyl-3-(3-hydroxyphenyl)propyl]morpholine (6). A mixture of *cis* and *trans*-2,6-dimethylmorpholine (7:3 cis/trans ratio) (7.5 g, 80 mmol) was added to a solution of (*R*)-(−)-5 (7.5 g, 27 mmol) in acetonitrile (150 mL). The reaction mixture was stirred at 50 °C for 10 h. The solvent was evaporated under vacuum, and the residue was dissolved in ether. This solution was washed with water, dried (Na_2SO_4), and evaporated. Chromatography on silica gel using hexane/ethyl acetate 95:5 as eluant afforded 1.3 g of (*R*)-(+)-*trans*-2,6-dimethyl-4-[2-methyl-3-(3-hydroxyphenyl)propyl]morpholine (6): $[\alpha]^{25}_D$ +5.2° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.3–6.6 (4 H, m), 4.2–3.9 (2 H, m), 2.9–2.6 (2 H, m), 2.5–2.0 (8 H, m), 1.25 (6 H, d), 0.85 (3 H, d). Anal. Calcd for $C_{16}H_{25}NO_2$: C, 73.0; H, 9.5. Found: C, 72.8; H, 9.45; and 3.1 g of (*R*)-(+)-*cis*-2,6-dimethyl-4-[2-methyl-3-(3-hydroxyphenyl)propyl]morpholine (6): $[\alpha]^{25}_D$ +0.7° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.2–6.5 (5 H, m), 3.9–3.4 (2 H, m), 2.9–2.7 (2 H, m), 2.3–1.9 (6 H, m), 1.7 (1 H, q), 1.25–1.05 (6 H, m), 0.9 (3 H, d). Anal. Found: C, 73.1; H, 9.44.

trans and cis-(S)-(−)-2,6-Dimethyl-4-[2-methyl-3-(3-hydroxyphenyl)propyl]morpholine (6). The above-described procedure, starting from (*S*)-(+)-5 (4.7 g, 50 mmol) afforded 0.81 g of (*S*)-(−)-*trans*-6, $[\alpha]^{25}_D$ −5.4° (*c* 1, $CHCl_3$), and 2.1 g of (*S*)-(−)-*cis*-6, $[\alpha]^{25}_D$ −0.7° (*c* 1, $CHCl_3$).

(*R*)-(+)-*cis-2,6-Dimethyl-4-[2-methyl-3-[3-(cyclopropylmethoxy)phenyl]propyl]morpholine* (1). (*R*)-(+)-*cis*-6 (3 g, 9 mmol) was added to a suspension of potassium carbonate (2 g, 14 mmol), potassium iodide (20 mg), and chloromethylcyclopropane (1.5 g, 17 mmol) in dimethylformamide (15 mL), and the mixture was stirred for 6 h at 80 °C. After evaporation of the solvent, the residue was dissolved in ether and washed with water. Chromatography on silica gel using hexane/ethyl acetate 95:5 as eluant afforded 2.6 g of (*R*)-(+)-*cis*-2,6-dimethyl-4-[2-methyl-3-[3-(cyclopropylmethoxy)phenyl]propyl]morpholine (1): $[\alpha]^{25}_D$ +4.1° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.3–6.6 (4 H, m), 3.8 (2 H, d), 3.75–3.5 (2 H, m), 2.7 (2 H, t), 2.3–1.8 (6 H, m), 1.7 (1 H, m), 1.3–1.0 (6 H, m), 0.85 (3 H, d), 0.6 (4 H, m). Anal. Calcd for $C_{20}H_{31}NO_2$: C, 75.71; H, 9.78. Found: C, 75.68; H, 9.75.

(*S*)-(−)-*cis-2,6-Dimethyl-4-[2-methyl-3-[3-(cyclopropylmethoxy)phenyl]propyl]morpholine* (1). The above-described procedure, starting from (*S*)-(−)-*cis*-6 (2 g, 6 mmol), afforded 1.6 g of (*S*)-(−)-*cis*-1, $[\alpha]^{25}_D$ −4.1° (*c* 1, $CHCl_3$).

(*R*)-(+)-*trans-2,6-Dimethyl-4-[2-methyl-3-[3-(cyclopropylmethoxy)phenyl]propyl]morpholine* (1). The above-described procedure, starting from (*R*)-(+)-*trans*-6 (1.3 g, 4 mmol), afforded 1.0 g of (*R*)-(+)-*trans*-1, $[\alpha]^{25}_D$ +6.15° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.3–6.7 (4 H, m), 4.0 (2 H, m), 3.8 (2 H, d), 2.9–2.7 (2 H, m), 2.4–1.8 (8 H, m), 1.3–1.1 (6 H, m), 0.85 (3 H, d), 0.6 (2 H, d), 0.4 (2 H, d).

(*S*)-(−)-*trans-2,6-Dimethyl-4-[2-methyl-3-[3-(cyclopropylmethoxy)phenyl]propyl]morpholine* (1). The above-described procedure, starting from (*S*)-(−)-*trans*-6 (0.8 g, 2.5 mmol), afforded 0.65 g of (*S*)-(−)-*trans*-1, $[\alpha]^{25}_D$ −6.3° (*c* 1, $CHCl_3$).

Bioassay. *In Vitro Activity.* Fungitoxicity tests were carried out in Petri dishes, with potato dextrose agar, by assessing the inhibition of mycelial radial growth of treated colonies with respect to that of untreated ones. All compounds were tested as dimethyl sulfoxide (DMSO) solutions, containing Tween 20 (2%), which were added to the agar medium in such amount as to keep the final DMSO concentration at 0.5%.

Activity on Plants. Activity against *E. graminis* and *H. teres* was evaluated on artificially infected, potted, wheat plants (cv. Irnerio) and barley plants (cv. Arna), respectively.

Wheat and barley plants were treated by sprinkling the leaves with the compounds being tested dissolved in a water/acetone solution containing 20% of acetone (v/v) and 0.3% Tween 20. Preventive treatments were carried out 1 day before artificial infection. Infection of wheat plants was carried out by spraying the leaves with an aqueous, spore suspension of *E. graminis* (200 000 conidia/mL). Infection of barley plants was carried out by spraying the leaves with an aqueous, spore suspension of mycelium homogenate of *H. teres* (10 g of filtered mycelium in 100 mL of water).

Inoculated plants were kept at 21 °C in a wet room for 24 h and then in a conditioned environment with a daylight period of 16 h and relative humidity of 70% for the incubation of the diseases: 12 days at 20 °C for *E. graminis*, 15 days at 23 °C for

Table I. Lipase-Catalyzed Resolution of 2

substrate	enzyme	solvent	conv, %	alcohol 2			ester 3		
				$[\alpha]^{25}_D$[a]	conf	ee,[b] %	$[\alpha]^{25}_D$[a]	conf	ee, %
3	PS	H_2O[c]	45	-3.1	S	30	-2.5	R	22
3	PPL	H_2O	45	+1.0	R	9	+0.4	S	3
2	PS	AcOEt[d]	55	+10.1	R	95	+9.6	S	84
2	PS	AcOEt	45	+8.4	R	79	+11.3	S	98
2	PPL	AcOEt	60	-1.6	S	15	-1.5	R	13

[a] (c 1, $CHCl_3$). [b] Determined by HPLC analysis using a Daicel Chiralcel OD column. [c] The hydrolytic reactions were performed in phosphate buffer 0.01 N, pH 7 (30 mL), substrate (1 g), and enzyme (100 mg) at 30 °C. [d] The transesterification reactions were performed in ethyl acetate (40 mL), substrate (1 g), and enzyme (100 mg) at 30 °C.

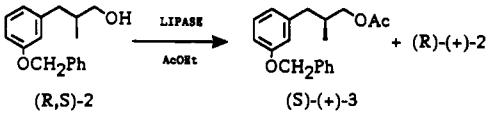

Figure 2. Lipase-catalyzed transesterification of (*R,S*)-2.

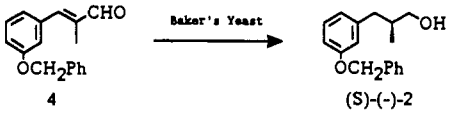

Figure 3. Microbial reduction of 4.

H. teres. At the end of the incubation periods, fungicidal activity was assessed as percent disease control with respect to infected but untreated plants.

RESULTS AND DISCUSSION

The key intermediate 2 was chosen as chiral starting material in the synthesis of optically active 1 both because it could be used as a precursor of a wide class of fungicides carrying different substitutions on the aromatic ring and because the phenol moiety could be easily deprotected. Optically active 2 was prepared by two different routes: the kinetic resolution of the alcohol 2, catalyzed by lipases, and the reduction of the aldehyde, 4, catalyzed by bakers' yeast.

To study the stereoselectivity of lipases, we compared the hydrolysis of the racemic ester 3, in water, to the transesterification of the racemic alcohol 2, in ethyl acetate. Two enzymes were tested, one of mammalian origin (porcine pancreatic lipase) and the other from a microbial source (lipase PS from *P. cepacia*). The hydrolytic reactions were carried out in phosphate buffer solution, pH 7 at 30 °C. The pH was kept constant by addition of 0.5 N NaOH, using a pH-stat. The reactions were stopped at different degrees of conversion, and the products were recovered as described under Experimental Procedures. Transesterification reactions were carried out in anhydrous ethyl acetate, which acted as both acylating agent and reaction medium. Powdered enzymes were added to a solution of (*R,S*)-2 in ethyl acetate at 30 °C, and the suspensions were shaken with an orbital shaker at 200 rpm. The reaction mixture was analyzed by gas chromatography and worked up as described under Experimental Procedures.

As reported in Table I, PPL showed very poor stereoselectivity in both reaction conditions. Lipase PS proved instead to be a much more selective catalyst in ethyl acetate than in water. The enantiomeric ratio values (*E*) (Chen et al., 1982) calculated in water and in ethyl acetate were 2.1 and 29, respectively, indicating a 14-fold increase in enantioselectivity. This surprising result confirms literature data reporting that enzyme regioselectivity and stereoselectivity can be dramatically affected by the reaction medium (Bianchi et al., 1990; Fitzpatrick and Klibanov, 1991). These findings can possibly be explained by the rigidity of the enzyme structure in an anhydrous organic solvent, which is higher than in water, where the increased conformational flexibility of the protein results in a relaxation of stereoselectivity.

Lipase PS-catalyzed transesterification (Figure 2) afforded the ester 3 in the *S* form and the alcohol 2 in the *R* form, both with optical purity >95%.

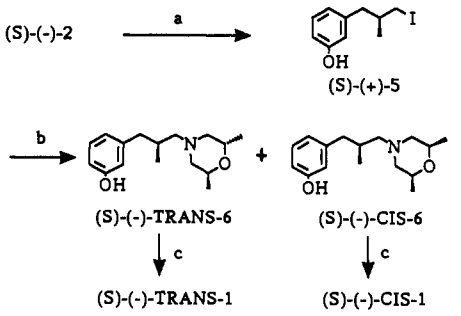

Figure 4. Synthetic route to optically active 1: (a) trimethylchlorosilane/NaI; (b) 2,6-dimethylmorpholine; (c) chloromethylcyclopropane/K_2CO_3.

In a second approach, we took advantage of the well-known ability of bakers' yeast to reduce carbon–carbon double bonds stereoselectively to prepare optically active 2 starting from the α,β-unsaturated aldehyde 4.

The reduction was performed by adding an ethanol solution of 4 to a suspension of bakers' yeast in an aqueous glucose solution. The mixture was incubated at 28 °C for 24 h, and the reaction products were recovered and purified as described under Experimental Procedures. The reaction (Figure 3) afforded (*S*)-(-)-2, with optical purity >95%, as the only product. No significant traces of byproducts, such as α,β-unsaturated alcohol or products of acyloin-type condensation, were detected (Bertolli et al., 1981).

The enantiomeric excess of optically active 2 was determined by HPLC. The absolute configuration of 2 was assigned by chemical correlation with the known (*S*)-(-)-2-methyl-3-phenyl-1-propanol (Cardillo et al., 1988) using the following procedure. The phenolic group of (*S*)-(+)-3 was deprotected (H_2/Pd–C), transformed into the corresponding mesylate (CH_3SO_2Cl/Py), and reduced (H_2/Pd–C); alkaline hydrolysis of the acetate gave (*S*)-(-)-2-methyl-3-phenyl-1-propanol [$[\alpha]^{25}_D$ -10.9° (*c* 1, C_6H_6) [lit. $[\alpha]^{25}_D$ -11.0° (*c* 1, C_6H_6)]], thereby confirming both the optical purity and the absolute configuration.

Enzymatically prepared (*S*)-(-)-2 was then used in the synthesis of optically pure 1 (Figure 4). Introduction of iodine and deprotection of the phenol group were carried out simultaneously by treating (*S*)-(-)-2 with trimethylchlorosilane and sodium iodide. The resulting intermediate (*S*)-(+)-5 was treated with 2,6-dimethylmorpholine (cis:trans isomers ratio 7:3) to give a mixture of (*S*)-(-)-*cis*-6 and (*S*)-(-)-*trans*-6. The two isomers were easily separated by silica gel chromatography.

(*S*)-(-)-*cis*-6 and (*S*)-(-)-*trans*-6 were converted into (*S*)-

Table II. EC_{50} Values of Racemate and Pure Enantiomers of 1 in Fungicidal in Vitro Tests

pathogen	EC_{50} values, mg/L					
	trans-(*R*,*S*)-1	*cis*-(*R*,*S*)-1	*trans*-(*R*)-1	*trans*-(*S*)-1	*cis*-(*R*)-1	*cis*-(*S*)-1
Botrytis cinerea	2.0	1.1	6.0	2.0	1.1	1.1
Cercospora beticola			70	20	4.5	6.0
Cercosporella herpotrichoides	1.0	0.1	2.3	1.0	0.1	0.1
Septoria nodarum			0.6	0.6	0.6	0.6
Helminthosporium oryzae			3.4	1.4	0.3	0.3
Rhizoctonia solani			200	120	40	60

Table III. EC_0 Values of Racemate and Pure Enantiomers of 1 in Fungicidal in Vivo Tests

pathogen (plant)[a]	EC_0 values, mg/L					
	trans-(*R*,*S*)-1	*cis*-(*R*,*S*)-1	*trans*-(*R*)-1	*trans*-(*S*)-1	*cis*-(*R*)-1	*cis*-(*S*)-1
E. graminis (wheat)	>500	130	>500	500	115	125
H. teres (barley)	400	68	500	20	24	74

[a] One-day protective application.

(−)-*cis*-1 and (*S*)-(−)-*trans*-1 by alkylating the phenol group with chloromethylcyclopropane and potassium carbonate.

(*R*)-(+)-*cis*-1 and (*R*)-(+)-*trans*-1 were prepared by the same sequence of reactions, starting from (*R*)-(+)-2.

The fungicidal activity of the pure stereoisomers of 1 was evaluated in comparison with the racemic cis and trans mixtures, both in vitro, against a number of fungi (Table II), and in vivo, against *E. graminis* and *H. teres* on wheat and barley plants, respectively (Table III).

As shown in Table II, the overall order of activity, based on EC_{50} values, is *R*-cis ≥ *S*-cis > *S*-trans > *R*-trans. This suggests that cis/trans isomerism on the morpholine ring plays the most important role in determining the fungitoxicity of the molecule. In most cases only a slight difference of activity is displayed by the *R* and *S* enantiomers.

Similar results were obtained from the in vivo test against *E. graminis* on wheat plants.

Conversely, a different behavior was observed in the control of *H. teres* on barley plants. In this case the *S*-trans isomer was found to be as active as the *R*-cis isomer, namely 25 times more active than the *R*-trans form (Table III). It is noteworthy that the activity of the racemic trans mixture is similar to the activity of the less effective *R*-trans isomer, suggesting an antagonistic effect, due to the preferential binding of such a stereoisomer to the target enzyme.

In conclusion, this combined chemoenzymatic approach provides a convenient method for the preparation of optically pure, chiral fungicides, in quantities suitable for an accurate study of their biological activity. The preparation of new chiral intermediates for the synthesis of optically active pesticides is currently under investigation.

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LITERATURE CITED

Bertolli, G.; Fronza, G.; Fuganti, C.; Grasselli, P.; Majori, L.; Spreafico, F. On the α-ketols Obtained from α,β-Unsaturated Aromatic Aldehydes and Bakers' Yeast. *Tetrahedron Lett.* **1981**, *22*, 965.

Bianchi, D.; Cesti, P. Lipase-Catalyzed Stereoselective Thiotransesterification of Mercapto Esters. *J. Org. Chem.* **1990**, *55*, 5657.

Bianchi, D.; Cesti, P.; Battistel, E. Anhydrides as Acylating Agents in Lipase-Catalyzed Stereoselective Esterification of Racemic Alcohols. *J. Org. Chem.* **1988**, *53*, 5531.

Bianchi, D.; Cesti, P.; Spezia, S.; Garavaglia, C.; Mirenna, L. Chemoenzymatic Synthesis and Biological Activity of Both Enantiomeric Forms of Tetraconazole, a New Antifungal Triazole. *J. Agric. Food Chem.* **1991**, *39*, 197.

Boland, W.; Frössl, C.; Lorenz, M. Esterolytic and Lipolytic Enzymes in Organic Synthesis. *Synthesis* **1991**, 1049.

Cardillo, G.; D'Amico, A.; Orena, M.; Sandri, S. Diastereoselective Alkylation of 3-Acylimidazolidin-2-ones: Synthesis of (R)- and (S)-Levandulol. *J. Org. Chem.* **1988**, *53*, 2354.

Chen, C. S.; Fulimoto, Y.; Girdaukas, G.; Sih, C. J. Quantitative Analyses of Biochemical Kinetic Resolutions of Enantiomers. *J. Am. Chem. Soc.* **1982**, *104*, 7294.

Fitzpatrick, P. A.; Klibanov, A. M. How can the Solvent Affect Enzymes Enantioselectively. *J. Am. Chem. Soc.* **1991**, *113*, 3166.

Francalanci, F.; Cesti, P.; Cabri, W.; Bianchi, D.; Martinengo, T.; Foa', M. Lipase-Catalyzed Resolution of Chiral 2-Amino-1-Alcohols. *J. Org. Chem.* **1987**, *52*, 5079.

Fuchs, A. Implications of stereoisomerism in agricultural fungicides. In *Stereoselectivity of Pesticides, Chemicals in Agriculture*; Welling, W., Ed.; Elsevier: Amsterdam, 1988; Vol. 1, pp 203–262.

Jones, J. B. Enzymes in Organic Synthesis. *Tetrahedron* **1986**, *42*, 3351.

Klibanov, A. M. Asymmetric Transformation Catalyzed by Enzymes in Organic Solvents. *Acc. Chem. Res.* **1990**, *23*, 114.

Laumen, K.; Schneider, M. P. A Highly Selective Ester Hydrolase from Pseudomonas Sp. for the Enzymatic Preparation of Enantiomerically Pure Secondary Alcohols; Chiral Auxiliaries in Organic Synthesis. *J. Chem. Soc., Chem. Commun.* **1988**, 598.

Pommer, E. H. Chemical Structure-Fungicidal Activity Relationships in Substituted Morpholines. *Pestic Sci.* **1985**, *15*, 285–295.

Servi, S. Bakers' Yeast as a Reagent in Organic Synthesis. *Synthesis* **1990**, 1.

Tombo, G. M.; Bellus, D. Chirality and Crop Protection. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1193.

Wang, Y. F.; Lalonde, J. J.; Momongan, M.; Bergbreiter, D. E.; Wong, C. H. Lipase-Catalyzed Irreversible Transesterification Using Enol Esters as Acylating Reagents: Preparative Enantio- and Regioselective Synthesis of Alcohols, Glycerol Derivatives, Sugars and Organometallics. *J. Am. Chem. Soc.* **1988**, *110*, 7200.

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Registry No. (*R*)-(+)-*cis*-1, 143238-98-6; (*S*)-(−)-*cis*-1, 143238-99-7; 1, 137217-11-9; (*S*)-(−)-2, 143239-00-3; (*R*)-(+)-2, 143239-01-4; (±)-2, 143291-83-2; (*S*)-(+)-3, 143239-02-5; (*R*)-(−)-3, 143239-03-6; (±)-3, 143291-84-3; 4, 134127-81-4; (*R*)-(−)-5, 143239-04-7; (*S*)-(+)-5, 143239-05-8; 6, 115195-51-2; (*S*)-(−)-*cis*-6, 143291-85-4; (*R*)-(+)-*cis*-6, 143291-86-5; 2,6-dimethylmorpholine, 141-91-3.