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Asymmetric Chemoenzymatic Synthesis of Miconazole and Econazole Enantiomers. The Importance of Chirality in Their Biological Evaluation

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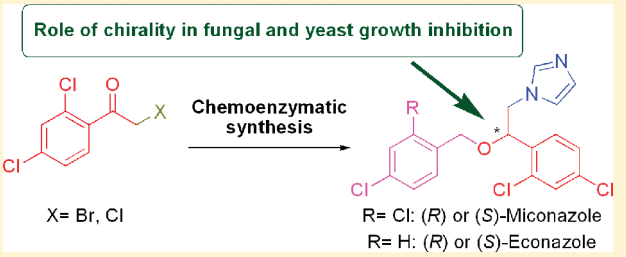
Supporting Information

ABSTRACT: A simple and novel chemoenzymatic route has been applied for the first time in the synthesis of miconazole and econazole single enantiomers. Lipases and oxidoreductases have been tested in stereoselective processes; the best results were attained with oxidoreductases for the introduction of chirality in an adequate intermediate. The behaviors of a series of ketones and racemic alcohols in bioreductions and acetylation procedures, respectively, have been investigated; the best results were found with alcohol dehydrogenases A and T, which allowed the production of (*R*)-2-chloro-1-(2,4-dichlorophenyl)ethanol in enantiopure form under very mild reaction conditions. Final chemical modifications have been performed in order to isolate the target fungicides miconazole and econazole both as racemates and as single enantiomers. Biological evaluation of the racemates and single enantiomers has shown remarkable differences against the growth of several microorganisms; while (*R*)-miconazole seemed to account for most of the biological activity of racemic miconazole on all the strains tested, both enantiomers of econazole showed considerable biological activities. In this manner, (*R*)-econazole showed higher values against *Candida krusei*, while higher values were observed for (*S*)-econazole against *Cryptococcus neoformans*, *Penicillium chrysogenum*, and *Aspergillus niger*.



INTRODUCTION

The preparation of enantiomerically pure compounds is a highly demanding task for the industrial sector because of the different activities of drug enantiomers in pharmaceutical and agrochemical applications.[1] As a result of advances in asymmetric organic synthesis and analytical separation technologies, efficient access to both single enantiomers of a racemate has been possible in many cases, allowing the study of their pharmacodynamic and pharmacokinetic properties. In fact, current requirements from regulatory authorities have largely increased the number of enantiomerically pure drugs rather than racemates presented for approval.[2]

Miconazole (**1a**) and econazole (**1b**) are antifungal agents which damage fungal organisms by interfering with ergoesterol biosynthesis. These compounds are usually employed in the treatment of vaginal diseases and several fungal infections in the skin of both human and animals (Figure 1), being well tolerated in clinical practice and showing biological activity shortly after application.[3] These two compounds correspond to the family of imidazole derivatives that also includes isoconazole, ketoconazole, sertaconazole, and sulconazole; all are well-known due to their potent activities against a variety of fungi. The therapeutic properties of their enantiomers and also of azole drugs[4] such as fluconazole, genaconazole, itraconazole, posaconazole, ravuconazole, and voriconazole usually differ, as occurs with sertaconazole[5] (**1c**), where the *R* enantiomer is 2 times more active than the racemic form against both fungi and yeasts.[6] This fact allows the administration of (*R*)-sertaconazole at doses half of those used for the racemic drug, suppressing minor risks of side effects and unspecific toxicities derived from the administration of the nonactive *S* enantiomer.

Asymmetric chemical transformations for access to this class of drugs or related chiral building blocks are generally based on stereoselective catalytic Henry reactions,[7] reductions of imidazole ketones[8] or α-halo ketones,[9] and also catalytic hydrosilylation reactions.[10] In this manner, the syntheses of miconazole and econazole single enantiomers have been scarcely reported, the synthesis of adequate chiral intermediates being made possible by the copper-catalyzed addition of nitromethane to 2,4-dichlorobenzaldehyde using a C_1-symmetric chiral aminopyridine ligand[6] or the enantioselective reduction of 2-chloro-1-(2,4-

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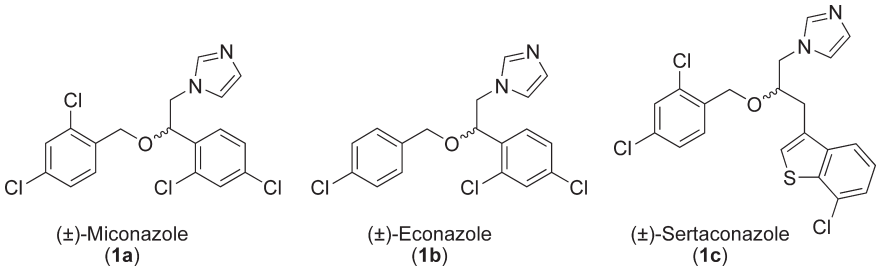


Figure 1. Structures of known antifungal agents such as miconazole, econazole, and sertaconazole.

dichlorophenyl)ethanone using a chiral oxazaborolidine.[9a] Alternatively, enantiomeric chromatographic separations of the corresponding racemates have been also successfully achieved.[11]

In our ongoing search toward the development of new stereoselective routes for the production of single-enantiomer drugs, herein we present an original chemoenzymatic approach for the production of enantiopure miconazole and econazole isomers. Initially the synthesis of adequate chiral building blocks will be described, and later two independent enzymatic approaches will be exhaustively investigated: (a) lipase-catalyzed kinetic resolution of racemic alcohols and (b) bioreduction of prochiral ketones using alcohol dehydrogenases. All efforts have been focused toward the production of enantiomerically pure precursors, which subsequently would be chemically modified in order to produce the desired drugs. The so-obtained chiral drugs as single enantiomers or racemic mixtures will both be tested in growth inhibition studies against different yeasts and fungi.

RESULTS AND DISCUSSION

Chemistry. Biocatalytic processes are challenging tools for organic chemists, offering new possibilities for the manufacture of pharmaceuticals under environmentally friendly conditions.[12] One of the main advantages of biocatalysis in comparison with conventional processes is based on the remarkable properties of the biological catalysts from a chemo-, regio-, and stereoselective point of view.[13] Additionally, enzymatic reactions can be carried out at ambient temperature and atmospheric pressure, avoiding the use of hazardous organic reagents and unwanted side reactions such as racemization and epimerization that frequently occur in base-mediated processes.[14] Without a doubt, the possibility of carrying out biocatalyzed stereoselective processes not only in water but also in media different from organic solvents, such as ionic liquids and supercritical fluids, has opened a window of opportunities for these "green" catalysts.[15] In addition, new possibilities have also recently arisen because of the production of new active and stable biocatalysts by using directed evolution methods,[16] improving protein expression systems and high-throughput screening methods.[17]

The use of lipases and alcohol dehydrogenases as responsible for the stereoselective catalytic step in a multistep synthetic route has been largely investigated.[18] Thus, lipases are the most common enzymes for the production of optically active alcohols and amines in organic solvents mainly by acylation reactions, while isolated alcohol dehydrogenases or microbial cells are the most useful biocatalysts for the bioreduction of ketones.

In this manner, we initially focused our attention on the development of asymmetric reactions for the stereoselective production of the alcohol **4**, the precursor of the antifungal imidazolium compounds targeted in this research (Scheme 1). With this in mind, the commercially available 2-bromo-1-(2,4-dichlorophenyl)ethanone (**2**) was transformed into the ketone **3** in 87% yield by reaction with imidazole in tetrahydrofuran (THF) as solvent and at room temperature. At this point the bioreduction of ketone 3 was undertaken using isolated alcohol dehydrogenases (ADHs), which have led to optimal results in the synthesis of a wide number of pharmaceuticals in aqueous medium.[19] We initially screened a number of already known oxidoreductases such as ADH T,[20] ADH LB,[21] ADH CP,[22] ADH PR2,[23] ADH RS1,[19] and ADH A,[24] but none of them showed even minimal activity toward the reduction of **3**.

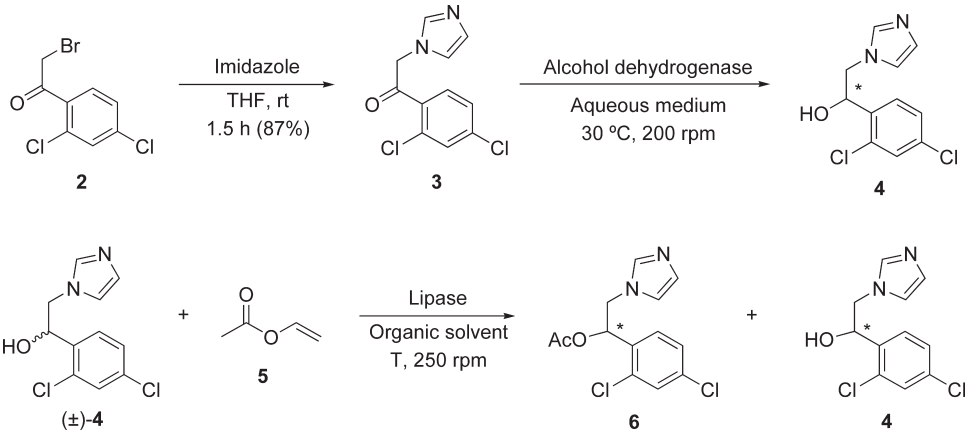
In view of this lack of activity we decided to turn our attention to the use of different enzymes, such as lipases, hydrolases active in both aqueous and organic medium. Then, the enzymatic acetylation of racemate **4** was studied, but unfortunately no reaction was observed at either 30 or 60 °C using vinyl acetate (VinOAc, **5**) as acyl donor, different organic solvents (THF, 1,4-dioxane, or the vinyl acetate itself), and a representative set of biocatalysts such as *Candida antarctica* lipase B (CAL-B), *Pseudomonas cepacia* lipase (PSL-C I), *Candida antarctica* lipase A (CAL-A), pancreas porcine lipase (PPL), *Candida cylindracea* lipase (CCL) or *Chromobacterium viscosum* lipase (CVL).

The negligible reactivity of all tested enzymes toward prochiral ketone **3** and racemic alcohol **4** caused us to look for an alternative and easily accessible chiral building block for the production of miconazole (**1a**) and econazole (**1b**). In this manner, taking advantage of the commercial availability of α-bromoacetophenone **2**, the ketone was first reduced using $NaBH_4$ affording the alcohol (±)-**7a** after 1 h in very high isolated yield (Scheme 2). Then we decided to study the lipase-mediated kinetic resolution of **7a** and commercially available 2-chloro-1-(2,4-dichlorophenyl) ethanol (**7b**).

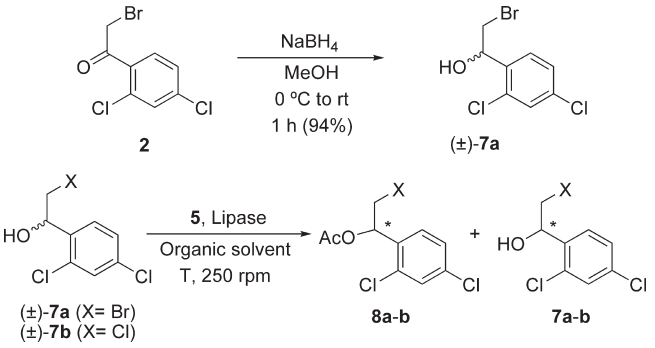
CAL-B did not show any activity toward the acetylation of alcohol **7a**; however, PSL catalyzed the kinetic resolution with a low conversion (2−3%) and excellent enantiopreference (>99% ee_P) when *tert*-butyl methyl ether (TBME) and vinyl acetate itself were used as solvents at 30 °C. Unfortunately, we have also observed very low reactivity with a less bulky substrate such as the racemic chlorinated derivative **7b**, obtaining optically active alcohol and acetate with similar conversion values and the alcohol (*S*)-**7b** in virtually racemic form; thus, no synthetically useful applications were attained.

At this point, the bioreduction of available α-halo ketones **2** and **9** were investigated using a set of commercially available purified alcohol dehydrogenases (Scheme 3). The most representative results are shown in Table 1. From the seven ADH species tested with the ketone **2**, only ADH T and ADH PR2 showed any activity; with ADH-T the (*R*)-alcohol was recovered in >99% yield and >99% ee (entry 1), and on the other hand (*S*)-**2a** was obtained in 37% yield and 72% ee using ADH PR2 as

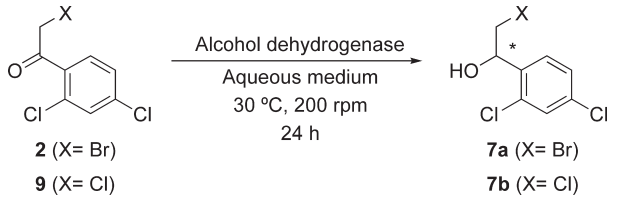
Scheme 1. Chemoenzymatic Synthesis of Alcohol 4 through Bioreduction of the Ketone 3 in Aqueous Medium or Lipase-Mediated Acetylation of the Racemate in Organic Solvents



Scheme 2. Chemical Synthesis and Lipase-Catalyzed Acetylation of Racemic Alcohols 7a,b Using Vinyl Acetate As Acyl Donor



Scheme 3. Bioreduction of α-Haloketones 2 and 9 in Aqueous Medium Using Different Alcohol Dehydrogenases



biocatalyst (entry 2). ADH T displayed a similar reactivity toward the ketone **9**, giving the alcohol (*R*)-**7b** in enantiomerically pure form without any traces of the initial ketone (entry 3). Other ADH species such as LB (entry 4) and PR2 (entry 5) led to the opposite enantiomer (*S*)-alcohol **7b** with a different grade of asymmetric induction; meanwhile ADH CP (entry 6), RS1 (entry 7), and A (entry 8) stereoselectively produced the *R* enantiomer, although from a synthetic point of view ADH A gave the most promising result, giving the alcohol in 74% yield.

Before the development of scalable processes, we wanted to investigate further improvements in the search for optimal

Table 1. Bioreduction of α-Halo Ketones 2 and 9 in Aqueous Systems at 30 °C and 250 rpm after 24 h

entry	ketone	ADH	ADH units[a]	*c* (%)[b]	ee_P (%)[c]	alcohol confign
1	**2**	T	3	>99	>99	*R*
2	**2**	PR2	3	37	72	*S*
3	**9**	T	3	>99	>99	*R*
4	**9**	LB	3	53	90	*S*
5	**9**	PR2	3	51	54	*S*
6	**9**	CP	3	<3	>99	*R*
7	**9**	RS1	3	44	>99	*R*
8	**9**	A	3	74	>99	*R*
9	**9**	T	1.5	65	>99	*R*
10	**9**	A	4.5	95	>99	*R*

[a] 1 unit (U) of ADH reduces 1.0 mM of acetophenone to 1-phenylethanol per minute at pH 7.5 and 30 °C in the presence of NAD(P)H. [b] Calculated by GC. [c] Determined by HPLC.

conditions in the bioreduction of ketone **9**. Thus, in the case of ADH T a lower amount of enzyme was employed, which led to a decrease in the conversion value to 65% after 1 day (1.5 units/3 mg of substrate, entry 9). On the other hand, 95% conversion was reached when a greater amount of ADH A was used (4.5 units, entry 10). Because of the fact that ADH A uses NADH as cofactor instead of the more expensive NADPH employed in ADH T catalyzed processes, the synthetic application was demonstrated when this process was scaled up with ADH A using a 20-fold amount of ketone, giving the same conversion and enantiomeric excess values as in the model reaction (see the Experimental Section). The absolute configuration of the so-obtained alcohol **7b** was demonstrated by comparison of its optical rotation value with that already described in the literature: $[\alpha]_D^{20} = -57.0$ (*c* 1, $CHCl_3$) (lit.[25] $[\alpha]_D^{20} = -59.1$ (*c* 1.2, $CHCl_3$)).

To emphasize the importance of this synthetic methodology, (*R*)-**7b** was transformed into enantiopure miconazole and econazole in a straightforward manner (Scheme 4). Therefore, the transformation of enantiopure alcohol (*R*)-**7b** into the corresponding imidazolium derivative (*R*)-**4** was initially carried out in a two-step sequence: first, basic cyclization of the halohydrin using sodium hydroxide (NaOH) gave the epoxide (*R*)-**10** in

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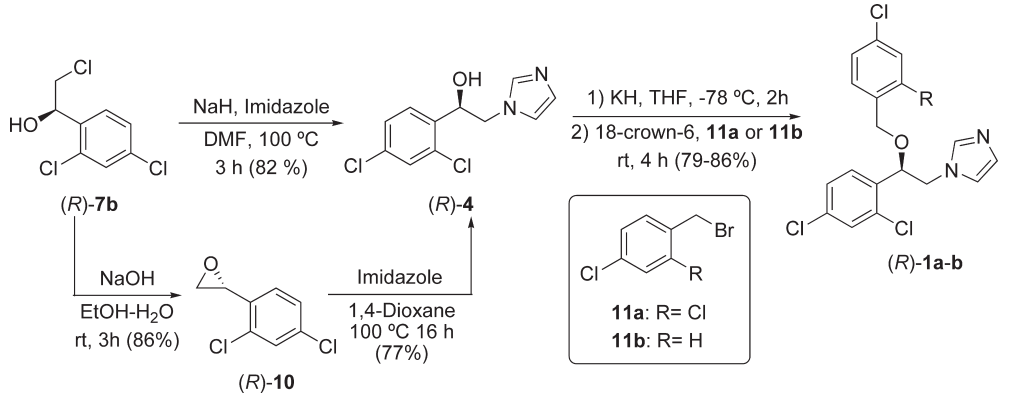
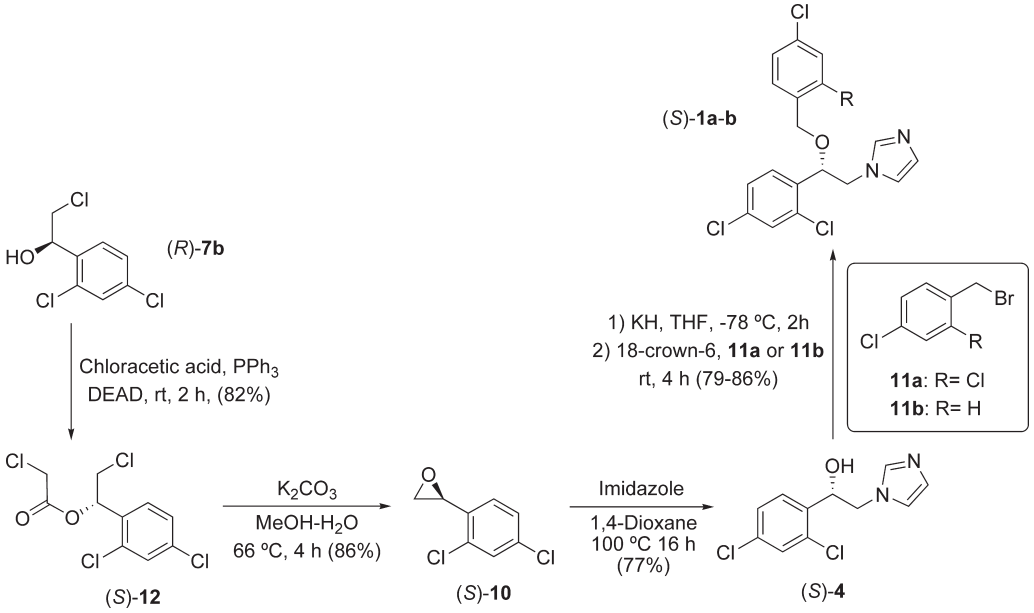
Scheme 4. Stereoselective Synthesis of (*R*)-Miconazole and (*R*)-Econazole from Enantiopure Alcohol (*R*)-7b



Scheme 5. Stereoselective Synthesis of (*S*)-Miconazole and (*S*)-Econazole from Enantiomerically Pure Alcohol (*R*)-7b



86% yield after 3 h at room temperature, and this compound was subsequently opened in a regioselective manner with imidazole in refluxing 1,4-dioxane to afford (*R*)-**4** in 77% yield without any loss of optical purity. Alternatively, because of the low boiling point detected for the epoxide intermediate that led to significant problems in its isolation, we successfully performed the nucleophile substitution of (*R*)-**7b** with imidazole using sodium hydride (NaH) and (*N*,*N*)-dimethylformamide (DMF) as solvent at 100 °C, giving the enantiopure alcohol (*R*)-**4** in 82% yield after just 3 h and purification through flash chromatography.

In both cases only the *R* enantiomer was detected by HPLC chiral analysis of the so-obtained products (see the Supporting Information). Additionally, the optical rotation values of the enantioenriched oxirane intermediate **10** and the alcohol **4** have been compared with previous data reported in the literature: oxirane (*R*)-**10**, $[\alpha]_D^{20} = -56.3$ (*c* 1, $CHCl_3$) (lit.[9b] $[\alpha]_D^{20} = -55.0$ (*c* 0.86, $CHCl_3$)); alcohol (*R*)-**4**, $[\alpha]_D^{20} = 77.5$ (*c* 1, $CHCl_3$) (lit.[9b] $[\alpha]_D^{20} = -79.0$ (*c* 0.51, $CHCl_3$) for (*R*)-**4** in 99% ee). Finally the alkylation of the free hydroxyl group was achieved by using potassium hydride (KH) as base, 18-crown-6, and the benzyl bromides **11a,b** to mediate the formation of both (*R*)-miconazole (**1a**) and (*R*)-econazole (**1b**) in 86% and 79% isolated yields, respectively, depending on the benzyl bromide derivative **11a** or **11b** used in the process.

Once we had efficiently performed the synthesis of (*R*)-**1a,b**, we decided to focus our efforts toward the synthesis of (*S*)-**1a,b** (Scheme 5). Unfortunately, none of the anti-Prelog ADHs tested allowed us to recover the alcohol (*S*)-**7b** in enantiopure form. In light of these results a different synthetic strategy starting from (*R*)-**7b**, prepared by enzymatic bioreduction with ADH A, and involving Mitsunobu inversion with chloroacetic acid, triphenylphosphine, and diethyl azodicarboxylate (DEAD) gave the ester (*S*)-**12** in 82% isolated yield.

Next (*S*)-**12** was deprotected with potassium carbonate, giving the epoxide (*S*)-**10** in 86% yield without any loss of the optical purity, and this compound was subsequently opened in a regioselective manner with imidazole in refluxing 1,4-dioxane to afford (*S*)-**4** in 77% yield without any loss of optical purity. In

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Table 2. In Vitro Susceptibilities of Target Microorganisms against Racemates and Enantiomers of Miconazole (1a) and Econazole (1b)[a]

microorganism	(±)-1a	(*R*)-1a	(*S*)-1a	(±)-1b	(*R*)-1b	(*S*)-1b
Candida krusei	3.84−1.92	1.92−0.96	48.06−24.03	3.14−1.57	3.14−1.57	6.55−3.28
Cryptococcus neoformans	3.36−1.68	1.68−0.84	24.03−12.02	2.10−1.05	4.19−2.10	2.10−1.05
Penicillium chrysogenum	7.21−3.60	3.60−1.87	21.03−16.34	1.00−0.50	2.36−1.18	1.05−0.52
Aspergillus niger	12.02−6.01	6.09−3.04	>48.06	2.10−1.08	8.73−4.36	2.10−1.05

[a] The MIC_{100} values (μM) are given as the concentration ranges for which no apparent growth is detected (higher values) and low growth is detected (lower values).

both cases only the *R* enantiomer was detected by HPLC chiral analysis of the so-obtained products (see the Supporting Information). Additionally the optical rotation values of the enantioenriched oxirane intermediate **10** and the alcohol **4** have been compared with previous data reported in the literature: oxirane (*S*)-**10**, $[\alpha]_D^{20} = +54.2°$ (*c* 1, $CHCl_3$) (lit.[9b] $[\alpha]_D^{20} = +56.0°$ (*c* 0.63, $CHCl_3$)); alcohol (*S*)-**4**, $[\alpha]_D^{20} = +78.4°$ (*c* 1, MeOH) (lit.[26] $[\alpha]_D^{20} = +83.8°$ (*c* 1, MeOH) for (*S*)-**4** in 91% ee). Finally, the alkylation of the free hydroxyl group was performed in a manner analogous to that previously used with the *R* enantiomers, giving (*S*)-miconazole (**1a**) and (*S*)-econazole (**1b**) in 86% and 79% isolated yields, respectively.

Biological Evaluation. Once miconazole and econazole were prepared as racemates and single enantiomers, they were all tested as inhibitors in the growth of a series of yeasts (*Candida krusei* and *Cryptococcus neoformans*) and filamentous fungi (*Penicillium chrysogenum* and *Aspergillus niger*), to determine their MIC values (see Table 2). On comparison of data from both racemates, miconazole (**1a**) showed a high activity toward the growth of yeasts and fungi, requiring lower fungicide concentration. The susceptibility for these drugs showed that both enantiomers of econazole (**1b**) seemed to be active against the target strains, while a very different behavior was observed for miconazole: the enantiomer (*S*)-**1a** showed almost no antifungal activity. The fact that most of the activity for (±)-miconazole resides in the enantiomer (*R*)-**1a** highlights the achievement obtained through the asymmetric synthetic route previously described. Thus, these experimental results, which are in accordance with previous inhibition studies toward other pathogenic fungi[9a] or the enzyme complex 17α-hydrolase/17.20-lyase ($P\text{-}450_{17}\alpha$),[27] encourage the use of asymmetrical synthesis for increasing the therapeutic suitability for this well-known antifungal drug. In the case of econazole the activity of (*S*)-**1b** was apparently higher (except for *C. krusei*) in comparison with (*R*)-**1b**. The main differences were observed for *Aspergillus niger*, the *S* enantiomer being around 3 times more active than the racemic mixture and the *R* enantiomer. Both enantiomers displayed good activities toward the growth of the corresponding microorganisms, leading to a synergy in the action of the racemic mixture, although the observed inhibition did not follow a completely additive rule.

CONCLUSIONS

In summary, a viable approach for the production of enantiopure single enantiomers of miconazole and econazole has been described: alcohol dehydrogenases A and T have shown excellent activities and stereodiscrimination values for the production of adequate chiral building blocks. Thus, (*R*)-2-chloro-1-(2,4-dichlorophenyl)ethanol was obtained and later chemically modified for the global synthesis of the desired *R* enantiomer drugs in enantiopure form. The aforementioned alcohol has been efficiently used for the production of the *S* enantiomers on the basis of the stereoinversion of the chiral center using Mitsunobu reaction conditions. This novel chemoenzymatic route offers clear advantages to those already described, without requiring the use of hazardous or toxic catalysts for the introduction of chirality. The biological activity of the synthesized racemates and single enantiomers have been studied toward the growth of *Candida krusei*, *Cryptococcus neoformans*, *Penicillium chrysogenum* and *Aspergillus niger*, resulting in very different MIC values for each enantiomer of miconazole and econazole. Thus, while the activity of the racemic miconazole mostly resides in the *R* enantiomer, the *S* enantiomer of econazole has shown higher inhibition values toward *Cryptococcus neoformans*, *Penicillium chrysogenum*, and specially *Aspergillus niger*. The experimental results highlight the importance of developing methods for asymmetric drug synthesis, presenting enzymes as ideal tools for the introduction of chirality.

EXPERIMENTAL SECTION[28]

Bioreduction of 2-Bromo-1-(2,4-dichlorophenyl)ethanone (2) and 2-Chloro-1-(2,4-dichlorophenyl)ethanone (9) with ADHs. Analytical separations and experimental protocols are given for the measurement of conversion values and enantiomeric excess. Analytical separation in HPLC: Chiralcel OJ-H *n*-hexane/2-propanol (95:5), 0.8 mL/min, 20 °C; t_R(ketone **2**) = 15.5 min; alcohol **7a**, $t_R(R)$ = 12.2 min, $t_R(S)$ = 10.2 min. Analytical separation in HPLC: Chiralpak IA column in *n*-hexane/2-propanol (95:5), 0.8 mL/min, 20 °C; t_R(ketone **9**) = 7.8 min; alcohol **7b**, $t_R(R)$ = 12.8 min, $t_R(S)$ = 11.0 min.

ADH T. In an Eppendorf tube were added the ketone **2** (5 mg, 0.0186 mmol) or **9** (5 mg, 0.0179 mmol), 37.5 μL of 2-propanol as cofactor regenerator, and 630 μL of TRIS-HBr 50 mM buffer of pH 7.5 for ketone **2** and TRIS-HCl 50 mM buffer of pH 7.5 for ketone **9**. Then NADPH (75 μL of a NADPH 10 mM solution in TRIS-HCl 50 mM pH 7.5) as cofactor and 3.85 μL of ADH T (3U) were added. The reaction mixture was shaken at 250 rpm and 30 °C for 24 h and extracted with EtOAc (2 × 500 μL), and the extracts were dried over Na_2SO_4.

ADH LB. In an Eppendorf tube were added the ketone **2** (5 mg, 0.0186 mmol) or **9** (5 mg, 0.0179 mmol), 37.5 μL of 2-propanol as cofactor regenerator, 75 μL of a 10 mM solution of $MgBr_2$ in TRIS-HBr 50 mM buffer of pH 7.5 for ketone **2** or 75 μL of a 10 mM solution of $MgCl_2$ in TRIS-HCl 50 mM buffer of pH 7.5 for ketone **9**, 560 μL of TRIS-HBr 50 mM buffer of pH 7.5 for ketone **2**, and TRIS-HCl 50 mM buffer of pH 7.5 for ketone **9**. Then NADPH (75 μL of a NADPH 10 mM solution in TRIS-HCl 50 mM pH 7.5) as cofactor and 1.3 μL of ADH LB (3U) were added. The reaction mixture was shaken at 250 rpm and 30 °C for 24 h and extracted with EtOAc (2 × 500 μL), and the extracts were dried over Na_2SO_4.

ADH PR2. In an Eppendorf tube were added the ketone **2** (5 mg, 0.0186 mmol) or **9** (5 mg, 0.0179 mmol), 37.5 μL of 2-propanol as

cofactor regenerator, 638 μL of TRIS-HBr 50 mM buffer of pH 7.5 for ketone **2**, and TRIS-HCl 50 mM buffer of pH 7.5 for ketone **9**. Then NADPH (75 μL of a NADPH 10 mM solution in TRIS-HCl 50 mM pH 7.5) as cofactor and 23 mg of ADH PR2 (3U) were successively added. The reaction mixture was shaken at 250 rpm and 30 °C for 24 h and extracted with EtOAc (2 × 500 μL), and the extracts were dried over Na_2SO_4.

ADH CP. In an Eppendorf tube were added the ketone **2** (5 mg, 0.0186 mmol) or **9** (5 mg, 0.0179 mmol), 37.5 μL of 2-propanol as cofactor regenerator, 628 μL of TRIS-HBr 50 mM buffer of pH 7.5 for ketone **2**, and TRIS-HCl 50 mM buffer of pH 7.5 for ketone **9**. Then NADH (75 μL of a NADH 10 mM solution in TRIS-HCl 50 mM pH 7.5) as cofactor and 7.5 μL of ADH CP (3U) were added. The reaction mixture was shaken at 250 rpm and 30 °C for 24 h and extracted with EtOAc (2 × 500 μL), and the extracts were dried over Na_2SO_4.

ADH RS1. In an Eppendorf tube were added the ketone **2** (5 mg, 0.0186 mmol) or **9** (5 mg, 0.0179 mmol), 6 μL of glucose dehydrogenase (3U) as cofactor regenerator, 10 mg of glucose, 658 μL of TRIS-HBr 50 mM buffer of pH 7.5 for ketone **2**, and TRIS-HCl 50 mM buffer of pH 7.5 for ketone **9**. Then NADH (75 μL of a NADH 10 mM solution in TRIS-HCl 50 mM pH 7.5) as cofactor and 10.9 μL of ADH RS1 (3U) were added. The reaction mixture was shaken at 250 rpm and 30 °C for 24 h and extracted with EtOAc (2 × 500 μL), and the extracts were dried over Na_2SO_4.

ADH A. In an Eppendorf tube were added the ketone **2** (5 mg, 0.0186 mmol) or **9** (5 mg, 0.0179 mmol), 112.5 μL of 2-propanol as cofactor regenerator, 462 μL of TRIS-HBr 50 mM buffer of pH 7.5 for ketone **2**, and TRIS-HCl 50 mM buffer of pH 7.5 for ketone **9**. Then NADH (75 μL of a NADH 10 mM solution in TRIS-HCl 50 mM pH 7.5) as cofactor and 100 μL of ADH A (100 μL of a solution of a 1 mg of pure ADH A in 0.6 mL of buffer, 3U) were successively added. The reaction mixture was shaken at 250 rpm and 30 °C for 24 h and extracted with EtOAc (2 × 500 μL), and the extracts were dried over Na_2SO_4.

Scale-Up of the Bioreduction of 2-Chloro-1-(2,4-dichlorophenyl)ethanone (9) with ADH-A. To a solution of 2-chloro-1-(2,4-dichlorophenyl)ethanone (**9**; 100 mg, 0.45 mmol) in a mixture of 2-propanol (2.68 mL) and TRIS-HCl 50 mM buffer of pH 7.5 (9.68 mL) were successively added NADH (1.79 mL of a NADH 10 mM solution in TRIS-HCl pH 7.5 buffer) and ADH A (3.75 mL of an ADH A solution (1.75 mg/mL) in TRIS-HCl pH 7.5 buffer). The reaction mixture was shaken at 250 rpm and 30 °C for 24 h and monitored by GC analysis. After this time the mixture was extracted with EtOAc (3 × 20 mL), the organic layers were combined, dried over Na_2SO_4, and filtered, and the solvent was removed by distillation under reduced pressure, affording 100 mg of (*R*)-**7b** as a white solid (99%) with enough purity to perform the next synthetic step without further purification.

2-Chloro-1-(2,4-dichlorophenyl)ethanol (7b). Yield: 89%. R_f (5% EtOAc/hexane): 0.31. Mp: 46−47 °C. IR (KBr): ν 3410, 3094, 2957, 1591, 1563, 1471, 1384, 1078, 1049, 869, 823, 782 cm^{-1}. ^1H NMR ($CDCl_3$, 300.13 MHz): δ 2.77 (d, 1H, $^3J_{HH}$ = 3.48 Hz), 3.52 (dd, 1H, $^2J_{HH}$ = 11.4 Hz, $^3J_{HH}$ = 8.4 Hz), 3.89 (dd, 1H, $^2J_{HH}$ = 11.4 Hz, $^3J_{HH}$ = 2.9 Hz), 5.26 (m, 1H), 7.31 (dd, 1H, $^3J_{HH}$ = 8.4 Hz, $^4J_{HH}$ = 2.0 Hz), 7.38 (d, 1H, $^4J_{HH}$ = 2.0 Hz), 7.58 (dd, 1H, $^3J_{HH}$ = 8.3 Hz). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 49.2, 70.3, 127.6, 128.6, 129.3, 132.5, 134.6, 135.9. MS (EI$^+$, *m*/*z*): 226 ($^{37}Cl^{35}Cl^{35}Cl$M$^+$, 48%), 224 ($^{35}Cl^{35}Cl^{35}Cl$M$^+$, 50%), 177 (($^{37}Cl^{35}Cl^{35}Cl$M − $CH_2{}^{35}Cl)^+$, 70%), 175 (($^{35}Cl^{35}Cl^{35}Cl$M − $CH_2{}^{35}Cl)^+$, 100%). HRMS (EI$^+$, *m*/*z*): calcd for $C_8H_7Cl_3O$ (M)$^+$ 223.9562, found 223.9559. $[\alpha]_D{}^{20}$ = −57° (*c* 1, $CHCl_3$) for the *R* enantiomer. Analytical separation (HPLC): Chiralcel OB-H *n*-hexane/2-propanol (97:3), 0.8 mL/min, 20 °C, $t_R(S)$ = 9.8 min, $t_R(R)$ = 13.4 min for lipase-mediated resolution and Chiralpak IA *n*-hexane/2-propanol (95:5), 0.8 mL/min, 20 °C, $t_R(R)$ = 11.3 min, $t_R(S)$ = 13.7 min, t_R(ketone) = 7.8 min for bioreduction.

Synthesis of (*S*)-2-Chloro-1-(2,4-dichlorophenyl)ethyl Chloroacetate (12). To a solution of (*R*)-**7b** (100 mg, 0.44 mmol) in dry THF (10 mL) were successively added chloracetic acid (84 mg, 0.89 mmol) and triphenylphosphine (233 mg, 0.89 mmol). The solution was cooled to 0 °C, and DEAD (162 mL, 0.89 mmol) was added. The mixture was stirred at room temperature for 3 h. After this time THF was removed by distillation at reduced pressure, giving a crude reaction mixture that was purified by flash chromatography (10% EtOAc/90% hexane), affording 110 mg of (*S*)-**12** as a colorless oil (82%). R_f (10% EtOAc/hexane): 0.42. IR (NaCl): ν 3095, 2959, 1748, 1592, 1475, 1372, 1225, 1076, 1056, 1032, 866, 823, 787 cm^{-1}. ^1H NMR ($CDCl_3$, 300.13 MHz): δ 3.76 (dd, 1H, $^2J_{HH}$ = 12.1 Hz, $^3J_{HH}$ = 7.2 Hz), 3.86 (dd, 1H, $^2J_{HH}$ = 12.1 Hz, $^3J_{HH}$ = 3.7 Hz), 4.18 (s, 2H), 6.38 (dd, 1H, $^3J_{HH}$ = 7.2 Hz, $^3J_{HH}$ = 3.7 Hz), 7.30 (dd, 1H, $^3J_{HH}$ = 8.4 Hz, $^4J_{HH}$ = 2.0 Hz), 7.38−7.43 (m, 2H, $^3J_{HH}$ = 5.1 Hz). ^{13}C NMR (75.5 MHz): δ 40.5, 44.6, 72.8, 127.5, 128.5, 129.6, 132.4, 132.9, 135.4, 165.8. MS (EI$^+$, *m*/*z*): 304 (($^{37}Cl^{37}Cl^{35}Cl^{35}Cl$M)$^+$, 10%), 302 (($^{37}Cl^{37}Cl^{35}Cl^{35}Cl$M)$^+$, 20%), 300 (($^{35}Cl^{35}Cl^{35}Cl^{35}Cl$M)$^+$, 17%), 177 (($^{37}Cl^{35}Cl^{35}Cl$M − $CH_2{}^{35}Cl)^+$, 70%), 175 (($^{35}Cl^{35}Cl^{35}Cl$M − $CH_2{}^{35}Cl)^+$, 100%). HRMS (EI$^+$, *m*/*z*): calcd for $C_{10}H_8Cl_4O_2$ (M)$^+$ 299.9278, found 299.9281. $[\alpha]_D{}^{20}$ = +32.6° (*c* 1, $CHCl_3$). Analytical separation (HPLC). Chiralcel OJ-H *n*-hexane/2-propanol (90:10), 0.8 mL/min, 30 °C, $t_R(S)$ = 10.7 min, $t_R(R)$ = 11.9 min.

Synthesis of (*R*)-2-(2,4-Dichlorophenyl)oxirane (10). To a solution of (*R*)-**7b** (50 mg, 0.22 mmol) in EtOH (400 μL) were successively added H_2O (250 μL) and NaOH (18 mg, 0.45 mmol). The mixture was stirred at room temperature for 3 h. After this time EtOH was removed by distillation at reduced pressure. The aqueous residue was extracted with Et_2O (3 × 5 mL), the organic phases were combined and dried over Na_2SO_4, and the solvent was removed by distillation under reduced pressure, giving a crude reaction mixture that was purified by flash chromatography (100% hexane), affording 36 mg of (*R*)-**10** as a colorless oil (86%). R_f (100% hexane): 0.24. IR (NaCl): ν 3091, 3059, 2992, 2916, 1595, 1563, 1479, 1379, 1247, 1100, 1052, 988, 880, 824 cm^{-1}. ^1H NMR ($CDCl_3$, 300.13 MHz): δ 2.62 (dd, 1H, $^2J_{HH}$ = 5.6 Hz, $^3J_{HH}$ = 2.5 Hz), 3.18 (dd, 1H, $^2J_{HH}$ = 5.5 Hz, $^3J_{HH}$ = 4.1 Hz), 4.14 (dd, 1H, $^3J_{HH}$ = 3.9 Hz, $^3J_{HH}$ = 2.4 Hz), 7.18 (d, 1H, $^3J_{HH}$ = 8.4 Hz), 7.25 (dd, 2H, $^3J_{HH}$ = 8.4 Hz, $^4J_{HH}$ = 1.9 Hz), 7.38 (d, 1H, $^4J_{HH}$ = 1.9 Hz). ^{13}C NMR (75.5 MHz): δ 49.6, 50.6, 126.6, 127.4, 128.9, 133.8, 134.0, 134.3. MS (EI$^+$, *m*/*z*): 190 ($^{37}Cl^{35}Cl$M$^+$, 18%), 188 ($^{35}Cl^{35}Cl$M$^+$, 30%), 159 ($C_7H_5{}^{35}Cl_2{}^+$, 42%), 153 (($^{35}Cl^{35}Cl$M − $^{35}Cl)^+$, 100%0. HRMS (EI$^+$, *m*/*z*): calcd for $C_8H_6Cl_2O$ (M)$^+$ 187.9796, found 187.9802. Analytical separation (GC): Chiralsil Rt-β-dexe, temperature program 110 °C then 2 °C/min until 160 °C, $t_R(R)$ = 18.5 min, $t_R(S)$ = 19.2 min.

Synthesis of (*S*)-2-(2,4-Dichlorophenyl)oxirane (10). To a solution of (*S*)-**12** (150 mg, 0.22 mmol) in MeOH (1.5 mL) were successively added H_2O (1.5 mL) and K_2CO_3 (137 mg, 0.99 mmol). The mixture was stirred at reflux for 3 h. After this time MeOH was removed by distillation at reduced pressure. The aqueous residue was extracted with CH_2Cl_2 (3 × 5 mL), the organic phases were combined and dried over Na_2SO_4, and the solvent was removed by distillation under reduced pressure, giving a crude reaction mixture that was purified by flash chromatography (100% hexane), affording 80 mg of (*S*)-**10** as a colorless oil (86%). $[\alpha]_D{}^{20}$ = +54.2° (*c* 1, $CHCl_3$).

Synthesis of (*R*)-1-(2,4-Dichlorophenyl)-2-(1*H*-imidazol-1-yl)ethanol (4). To a solution of (*R*)-**10** (288 mg, 1.52 mmol) in 1,4-dioxane (663 μL) was added imidazole (125 mg, 1.83 mmol), and the mixture was stirred at 100 °C for 16 h. After this time the solvent was removed by distillation under reduced pressure, giving a crude reaction mixture that was purified by flash chromatography (5% MeOH/95% CH_2Cl_2), affording 300 mg of (*R*)- or (*S*)-**4** as a yellow solid (77%). R_f (5% MeOH/CH_2Cl_2): 0.26. Mp: 131−133 °C. IR (NaCl): ν 3320, 2956, 1590, 1325 cm^{-1}. ^1H NMR (CD_3OD, 300.13 MHz): δ 4.28−4.51 (m, 2H), 5.42−5.47 (m, 1H), 7.09 (s, 1H), 7.21 (s, 1H), 7.49−7.73

(m, 4H). ^{13}C NMR (CD_3OD, 75.5 MHz): δ 53.5, 70.7, 121.8, 128.8 (2C), 130.2, 130.4, 133.7, 135.4, 139.3, 139.6. MS (ESI$^+$, m/z): 259 (($^{35}Cl^{37}Cl$M + H)$^+$, 64%), 257 (($^{35}Cl^{35}Cl$M + H)$^+$, 100%). HRMS (ESI$^+$, m/z): calcd for $(C_{11}H_{11}Cl_2N_2O)^+$ $(M+H)^+$ 257.0243, found 257.0242. (*R*)-**4** $[\alpha]_D^{20}$ = −77.5° (*c* 1, $CHCl_3$). (*S*)-**4** $[\alpha]_D^{20}$ = +78.4° (*c* 1, MeOH). Analytical separation (HPLC): Chiralcel IA *n*-hexane/2-propanol (85:15), 0.8 mL/min, 20 °C, t_R(*R*) = 9.4 min, t_R(*S*) = 11.9 min.

Synthesis of (*R*)-1-(2-(2,4-Dichlorobenzyloxy)-2-(2,4-dichlorophenyl)ethyl)-1-*H*-imidazole (Miconazole, 1a).[23] To a solution of (*R*)-**4** (100 mg, 0.39 mmol) in dry THF (5.5 mL) at −78 °C under a nitrogen atmosphere was added a 30% dispersion of KH in mineral oil (71 mg, 0.53 mmol). The resulting suspension was stirred for 2 h at −78 °C. After this time, 18-crown-6 (103 mg, 0.39 mmol) and 1-(bromomethyl)-2,4-dichlorobenzene (**11a**; 187 mg, 0.78 mmol) were successively added. Then, the mixture was stirred for 4 h at room temperature, and after this time the reaction was quenched with H_2O (10 mL), the aqueous solution was extracted with CH_2Cl_2 (3 × 15 mL), the organic phases were combined, dried over Na_2SO_4, and filtered, and the solvent was removed by distillation under reduced pressure, giving a crude reaction mixture that was purified by flash chromatography (eluent gradient 100% CH_2Cl_2 to 5% MeOH/95% CH_2Cl_2), affording 127 mg of (*R*)-**1a** as a viscous oil (79%). R_f (5% MeOH/CH_2Cl_2): 0.31. IR (NaCl): ν 3194, 3113, 2932, 2883, 1590, 1563, 1506, 1471, 1383, 1232, 1094, 1043, 866, 820 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 4.08 (dd, 1H, $^2J_{HH}$ = 14.5 Hz, $^3J_{HH}$ = 7.4 Hz), 4.24 (dd, 1H, $^2J_{HH}$ = 14.5 Hz, $^3J_{HH}$ = 2.7 Hz), 4.35 (d, 1H, $^2J_{HH}$ = 12.6 Hz), 4.50 (d, 1H, $^2J_{HH}$ = 12.6 Hz), 5.03 (dd, 1H, $^3J_{HH}$ = 7.4 Hz, $^3J_{HH}$ = 2.7 Hz), 6.91 (s, 1H), 7.03 (s, 1H), 7.15−7.46 (m, 7H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 51.1, 68.1, 77.4, 119.6, 127.2, 127.8, 128.2, 129.1 (2C), 129.5, 129.8, 133.1, 133.2, 133.6 (2C), 134.2, 134.9, 137.7. MS (EI$^+$, m/z): 418 ($^{37}Cl^{37}Cl^{35}Cl^{35}ClM^+$, 14%), 416 ($^{37}Cl^{35}Cl^{35}Cl^{35}ClM^+$, 28%), 414 ($^{35}Cl^{35}Cl^{35}Cl^{35}ClM^+$, 23%), 335 ($^{37}Cl^{35}Cl^{35}Cl^{35}Cl$M − $CH_2C_3H_2N_2^+$, 37%), 161 ($C_7H_5{}^{37}Cl^{35}Cl^+$, 65%), 159 ($C_7H_5{}^{35}Cl_2^+$, 100%). HRMS (ESI$^+$, m/z): calcd for $(C_{18}H_{15}Cl_4N_2O)^+$ $(M+H)^+$ 414.9933, found 414.9949. (*R*)-**1a** $[\alpha]_D^{20}$ = −65.0° (*c* 1, CH_2Cl_2). (*S*)-**1a** $[\alpha]_D^{20}$ = +68.7° (*c* 1, CH_2Cl_2). Analytical separation (HPLC). Chiralcel OJ-H *n*-hexane/2-propanol (85:15), 0.8 mL/min, 40 °C, t_R(*R*) = 21.6 min, t_R(*S*) = 28.8 min.

Synthesis of (*R*)-1-[2-(4-Chlorobenzyloxy)-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole (Econazole, 1b). To a solution of (*R*)-**4** (49 mg, 0.19 mmol) in dry THF (2.2 mL) at −78 °C under a nitrogen atmosphere was added a 30% dispersion of KH in mineral oil (33 mg, 0.26 mmol). The resulting suspension was stirred for 2 h at −78 °C. After this time 18-crown-6 (51 mg, 0.19 mmol) and 1-(bromomethyl)-4-chlorobenzene (**11b**; 117 mg, 0.57 mmol) were successively added. Then the mixture was stirred overnight at room temperature. The reaction was quenched with H_2O (5 mL), the aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL), the organic phases were combined, dried over Na_2SO_4, and filtered, and the solvent was removed by distillation under reduced pressure, giving a crude reaction mixture that was purified by flash chromatography (eluent gradient 100% CH_2Cl_2 to 5% MeOH/95% CH_2Cl_2), affording 62 mg of (*R*)-**1b** as a viscous oil (86%). R_f (2% MeOH/CH_2Cl_2): 0.29. IR (NaCl): ν 3111, 3066, 2935, 2869, 1590, 1561, 1492, 1470, 1090, 1044, 821 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 4.01 (dd, 1H, $^2J_{HH}$ = 14.5 Hz, $^3J_{HH}$ = 7.7 Hz), 4.17 (m, 2H), 4.41 (d, 1H, $^2J_{HH}$ = 11.8 Hz), 4.94 (dd, 1H, $^3J_{HH}$ = 7.6 Hz, $^3J_{HH}$ = 2.7 Hz), 6.87 (s, 1H), 6.98−7.07 (m, 3H), 7.23−7.35 (m, 4H), 7.40−7.47 (m, 2H). ^{13}C NMR (75.5 MHz): δ 51.3, 70.7, 76.8, 119.7, 127.9, 128.4, 128.6 (2C), 128.9 (2C), 129.2, 129.6, 133.3, 133.8, 133.9, 134.9, 135.3, 137.8. MS (EI$^+$, m/z): 384 ($^{37}Cl^{37}Cl^{35}Cl$M$^+$, 10%), 382 ($^{37}Cl^{35}Cl^{35}Cl$M$^+$, 32%), 380 ($^{35}Cl^{35}Cl^{35}Cl$M$^+$, 33%), 257 ($C_{11}H_9{}^{37}Cl\,{}^{35}ClN_2O^+$, 65%), 255 ($C_{11}H_9{}^{35}Cl_2N_2O^+$, 100%). HRMS (ESI$^+$, m/z): calcd for $(C_{18}H_{16}Cl_3N_2O)^+$ $(M+H)^+$ 381.0323, found 381.0333. (*R*)-**1b** $[\alpha]_D^{20}$ = −88.5° (*c* 1, CH_2Cl_2). (*S*)-**1b** $[\alpha]_D^{20}$ = +87.1° (*c* 1, CH_2Cl_2). Analytical separation (HPLC). Chiralcel OJ-H *n*-hexane/2-propanol (85:15), 0.8 mL/min, 40 °C, t_R(*R*) = 20.5 min, t_R(*S*) = 34.3 min.

Biological Evaluation. The antimicrobial activities of the corresponding racemic mixtures and single enantiomers were performed using the microdilution method, as recommended in documents M27-A for yeast[29] and M38-P for filamentous fungi,[30] using 96-well microtiter plates. The sensitivities to the antimicrobial compounds were checked by visual inspection of the microbial growth on each well, after 24 h of incubation for *C. krusei* or 48 h for *C. neoformans*, *P. chrysogenum*, and *A. niger*. The given values were the average from six independent experiments, with 20% standard deviation between them (see Table 2).

■ ASSOCIATED CONTENT

Supporting Information. Text and figures giving details of the HPLC and GC methods and full characterization data for all novel organic compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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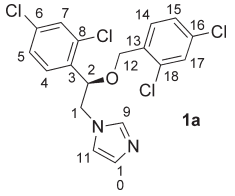
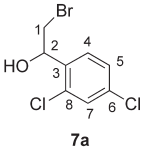
(28) (*R*)-**1a** Miconazole (**1a**) and 2-bromo-1-(2,4-dichlorophenyl) ethanol (**7a**) are given as examples for the assignments of numerical locants used for NMR assignment:





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