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Chemical and chemoenzymatic routes to 1-(benzothiazol-2-ylsulfanyl)-3-chloropropan-2-ol, a precursor of drugs with potential β-blocker activity

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Abstract

Several methods have been developed to prepare 1-(benzothiazol-2-ylsulfanyl)-3-chloropropan-2-ol **2** with good to high enantiomeric excess: 70 and 93% ees have in fact been obtained by baker's yeast-induced asymmetric reduction of the ketone precursor **1** and by kinetic resolution performed in the presence of lipase from *Pseudomonas* sp. ($E=38$), respectively. Compounds (*R*)-(+)-**2** and (*S*)-(–)-**2** have also been prepared by a chemical method in 90% yield and with enantiomeric excesses of 98 and 96.4%, respectively. HPLC on Chiralcel OD column separation of enantiomers (separability factor $\alpha=1.64$) has also been successfully performed. Compound **2** could, in turn, be used for the synthesis in an optically active form of various molecules, including β-aminoalcohols **6**, drugs with potential β-blocker activity.

1. Introduction

The increasing incidence of cardiovascular diseases led to the development of new drugs, molecules having mainly an adrenergic activity and, in particular, a β-blocking action.[1] Up to now three main β-adrenoceptors are known: β_1, β_2, and the rather recently identified β_3 (often classified as atypical β-adrenoceptors, namely those having low affinities and potencies towards β-adrenoceptor antagonists).[2,3] Drugs actually used in therapy and/or commercially available as β_1- and β_2-blockers are compounds with a well-defined structure. They are all chiral molecules (optically active or racemic) in which three functions can be identified: an aryl linked through -OCH_2-

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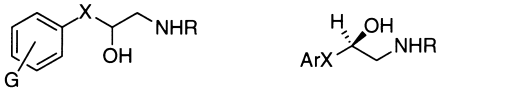
(or another group) to a moiety containing both an alcohol and an amino function, the latter bearing an *i*-propyl, *t*-butyl or a more bulky residue (Fig. 1). Almost all of them have X = -OCH_2- as the linking group of the aromatic ring. Other groups (-CH=CH-, -SCH_2-, -CH_2-CH_2-, -$NHCH_2$-) have also been tested, although with lower activity.[1]



a **b:** Biologically active enantiomer

X= -OCH_2-, -CH=CH-, -SCH_2-, -CH_2CH_2-, -$NHCH_2$-.

Figure 1.

On the other hand, concerning the aromatic part, some compounds in which the aryl has been replaced by a heteroaromatic group (X still being -OCH_2-) have also been prepared (pindolol, timolol, and tazolol). In such cases the introduction of a heterocycle offers many advantages over the carbocycles (e.g., the possibility of insertion of elements capable of giving additional interactions).[4]

Recently, during our studies to explore the synthetic potential of molecules containing the benzothiazole-2-thiolic group, we have been concerned with the synthesis of compounds having such a group linked to a residue similar to that present in the molecules in Fig. 1 (see Fig. 2), obtaining high ee values by baker's yeast-induced asymmetric reduction of the corresponding α-ketosulfides.[5]

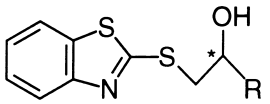
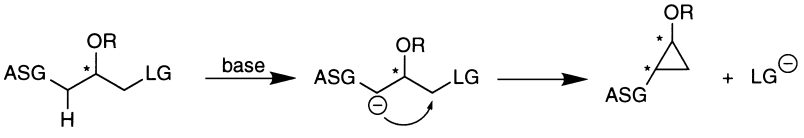


Figure 2.

Thus, since the molecules in Fig. 1 containing the benzothiazole moiety as the aromatic part had never been synthesized and tested for pharmacological activity until now, we decided to turn our attention to these, starting from the synthesis in enantiomerically pure (or highly enriched) form (by baker's yeast-induced asymmetric reduction of the corresponding alkanone or by other methods) of 1-(benzothiazol-2-ylsulfanyl)-3-chloropropan-2-ol **2** as a precursor. Besides, the thus obtained compound **2** could also be a new versatile C3 synthon, a potential precursor of a number of other optically active molecules (e.g., by the asymmetric synthesis depicted in Scheme 1).[6]



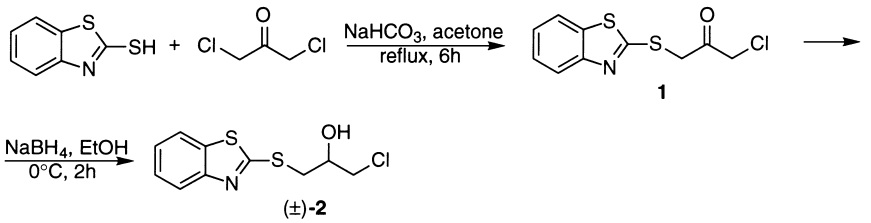
ASG (Anion-Stabilizing Group) = benzothiazole-2-thiol
LG (Leaving Group) = Cl

Scheme 1.

In this paper several routes to optically active **2** (ee up to 98%), as well as the possibility of conversion of **2** into the aminoalcohol **6** (with molecular features of a potential β-blocker), are reported.

2. Results and discussion

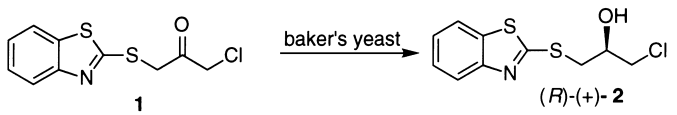
Reduction with $NaBH_4$ of 1-(benzothiazol-2-ylsulfanyl)-3-chloropropan-2-one **1**, obtained in turn from the benzothiazole-2-thiol and 1,3-dichloropropanone, affords (±)-1-(benzothiazol-2-ylsulfanyl)-3-chloropropan-2-ol **2** (Scheme 2).



Scheme 2.

On the other hand, the asymmetric reduction of **1** could in principle be performed using a variety of both biochemical and chemical methods.

As mentioned above, similar substrates having an alkyl group, such as CH_3, C_2H_5, C_4H_9 or C_6H_{11} instead of the $-CH_2Cl$ group, have already been asymmetrically reduced by us by baker's yeast, and the enantiomeric excesses obtained ranged between 60 and >99%.[5] Hence, with the aim of preparing optically active **2** with high enantiomeric excess, we (as a first approach) extended the same methodology to the reduction of **1** (Scheme 3).



Scheme 3.

The reduction was performed in the presence of baker's yeast and under a variety of conditions. Different ratios for substrate/yeast/sugar in tap water, phosphate buffer, hexane, and hexane/water were evaluated, as well as the addition of co-solvents (i.e., EtOH and DMSO) or CuO, an enzymatic cofactor which maximizes the oxidoreductase activity.[7,8]

In Table 1 a summary of the data obtained using an aqueous medium is reported. The enantiomeric excesses obtained, 26–65%, were strongly dependent upon the experimental conditions used. The highest enantiomeric excess was obtained using a ratio substrate:yeast = 1:16, glucose:substrate = 6.5, and yeast:glucose = 2.5. Addition of CuO did not affect the enantioselectivity (entry 9 versus entry 6, with ee = 54 and 58%, respectively).

A slight improvement of enantioselectivity was obtained when the reduction was conducted in hexane/water (ee = 70%, Table 2). This could also be due to a different conformation of the enzyme catalyzing the reduction in the organic solvent. However, another notable advantage of carrying

Table 1
Effect of the reaction conditions on the selectivity in reduction of the 1-(benzothiazol-2-ylsulfanyl)-3-chloropropan-2-one **1** using baker's yeast in water[a]

Entry	Yeast/Sugar	Additive	Time (h)	pH	Ee (%)[b]	Yield (%)[c]
1	-	-	5	7	26	30
2	-	EtOH[d]	2	7	54	25
3	-	EtOH[d]	22	7	54	30
4	2.5 (sucrose)	-	5	7	48	21
5	2.5 (sucrose)	EtOH[d]	2	7	46	20
6	2.5 (glucose)	-	2	7	58	32
7	2.5 (glucose)	-	48	7	65	30
8	2.5 (glucose)	EtOH[d]	2	7	48	29
9	2.5 (glucose)	CuO[e]	6	7	54	20
10[f]	1.2 (glucose)	Phosphate buffer/ EtOH-DMSO[g]	15	6	40	26

a) Substrate/yeast = 1/16, and glucose/substrate= 6.5; b) enantiomeric excesses were determined on the acetyl derivatives via HPLC (isocratic, flow rate 1.0 ml/minute, mobile phase: hexane/*i*-PrOH= 98/2); c) yields refer to the product isolated by chromatography; d) the ratio H_2O-EtOH is 10/1; e) Substrate/ CuO= 8/2; f) substrate/yeast = 1/24; g) buffer/additive = 95/2.5-2.5.

Table 2
Reduction of the 1-(benzothiazol-2-ylsulfanyl)-3-chloropropan-2-one **1** in the presence of baker's yeast in organic solvent and at room temperature

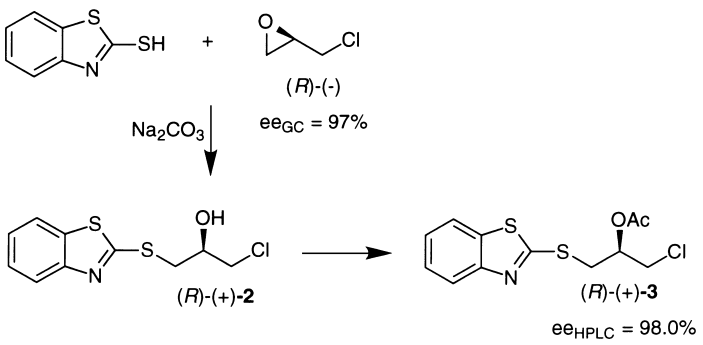
Entry	Medium	Time (h)	Yield (%)[a]	ee (%)[b]
1	hexane	48	61	64
2	hexane/water	48	68	70

a) yields refer to the product isolated by chromatography; b) enantiomeric excesses were determined on the acetyl derivatives via HPLC (isocratic, flow rate 1.0 ml/minute, mobile phase: hexane/*i*-PrOH= 98/2).

out the reduction in hexane is also the ease with which the product and eventually the remaining unreacted substrate are recovered from the reaction medium, which is reflected in the apparently higher observed chemical yields (see Tables 1 and 2). A similar observation has previously been made for other reductions using baker's yeast in organic solvents (e.g., toluene, benzene, carbon tetrachloride, ethyl ether, etc.).[9]

On the other hand, no effect was observed in the presence of selective enzyme inhibitors (e.g., allyl alcohol) while it has been proved to be successful in some cases.[10] The relative concentration of active (*R*)- and (*S*)-enzyme can depend, in fact, on the inhibitor concentration [(*R*)- and (*S*)-enzyme are selectively inhibited, e.g., by low and high concentrations of allyl alcohol, respectively].[11] In our case, however, allyl alcohol (12.5, 62.5, and 100 μl: see the Experimental section) added to the fermentation broth had no marked effect on the enantioselectivity.

The absolute configuration of the chlorohydrin obtained by yeast reduction was established as (*R*), based on a comparison of its specific rotation with that of an authentic sample prepared as depicted in Scheme 4. The same chlorohydrin (*R*)-(+)-**2** was in fact synthesized starting from the benzothiazole-2-thiol and the commercially available (*R*)-(–)-epichlorohydrin [(*S*)-(–)-**2**



Scheme 4.

was instead prepared from (*S*)-(+)-epichlorohydrin]. In these cases, together with high chemical yields (90%), complete maintenance of enantiomeric excess values of the starting epichlorohydrins (97%) was also observed in the obtained chlorohydrins **2**, so that the procedure depicted in Scheme 4 constitutes, in turn, another excellent approach to both (*R*)-(+)- and (*S*)-(–)-**2**.

The observed stereochemical outcome of baker's yeast-reduction of ketone **1** can be explained applying Prelog's rule.[12–16] The hydrogen is selectively added to the *Re*-face of the prochiral ketone, with reference (as predicted by the rule) to the actual size priorities of the groups (Fig. 3) and not to priorities as usually established (i.e., on the basis of atomic numbers of atoms). According to Prelog's rule, -CH_2Cl and 2-Btz-S-CH_2- are in fact the small and large groups,[17] respectively, and the hydrogen attack on the so-defined *Re*-face should then afford (as actually observed) just the (*R*)-alcohol (the absolute configuration being instead assigned in the latter on the basis of the common Cahn–Ingold–Prelog rules of priorities).

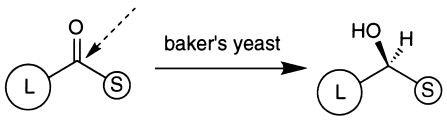
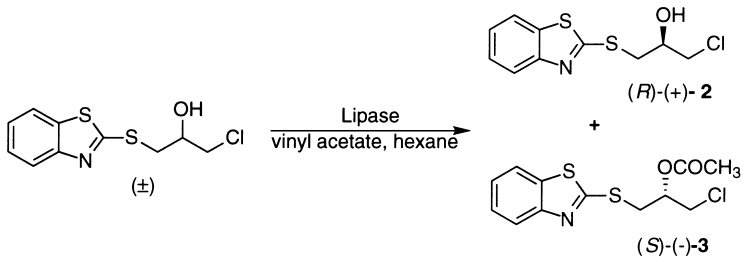


Figure 3. Prelog's rule for predicting the stereochemistry of alcohols formed by baker's yeast-mediated reduction of the corresponding prochiral ketones

Finally, concerning the use of optically inactive molecules as starting materials, a kinetic resolution of racemic-**2** by *O*-acylation performed in the presence of lipases was also explored. Lipase-catalyzed *O*-acylation has previously been proved to be a useful tool for the enantiomer separation of racemic 1-aryloxychlorohydrin precursors of aryloxypropanolamines.[18,19] This kinetic resolution accomplished by lipase-catalyzed transesterification was found to be highly enantioselective for chlorohydrins having a variety of substituted aromatic moieties.[19]

Therefore, we decided to extend the same procedure to the kinetic resolution by lipase-catalyzed transesterification of racemic **2**. A screening was performed to find the best lipase(s) able to catalyze the transesterification reaction between **2** and vinyl acetate (Scheme 5).[20] The results of the kinetic resolution of (±)-**2** using lipases from *Pseudomonas* sp., *Pseudomonas fluorescens*, *Candida cylindracea*, and lipase G are reported in Table 3.



Scheme 5.

Table 3
Lipase-catalyzed acylation of chlorohydrin (±)-**2**

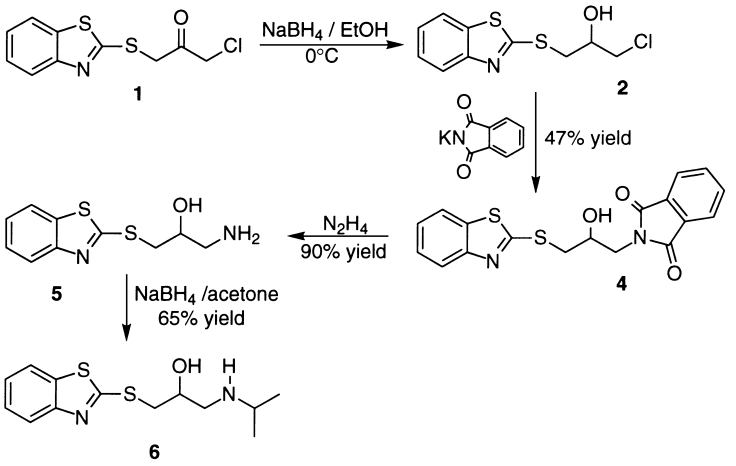
Lipase (source)	Substrate/Lipase[a]	Time (h)	c[b] (%)	ee (%) Alcohol[c]	ee (%) Ester[d]	E[e]
Pseudomonas fluorescens	1/1	4	31	42	93	43
"	1/5	4	42	58	78	15
Pseudomonas sp.(AK)	1/1	5	21	25	93	38
"	1/1	8	39	58	91	38
"	1/5	5	63	33	85	17
Candida cylindracea	1/5	8	39	1	6	1.1
lipase G	1/5	24	14	9	54	1.5

a) Substrate/lipase = w/w; b) c=conversion determined by HPLC; c) calculated according to the following equation: $c = ee_S/(ee_S+ee_P)$, where ee_S is the enantiomeric excess of remaining unreacted alcohol and ee_P is the enantiomeric excess of the produced ester; d) enantiomeric excess determined by HPLC; e) E= enantioselectivity factor.

These data show that (*S*)-(–)-**3**, and from this (*S*)-(–)-**2**, can in this case be prepared with enantiomeric excesses up to 93%. Presumably, even higher values could also be expected by stopping the reaction at lower conversions or by recycling the chlorohydrin obtained from the optically active **3** after its treatment with K_2CO_3 in aq. MeOH. Furthermore, higher ee values might also be obtained if pure rather than crude lipase powder was used (the latter could contain, in fact, more than one hydrolytic enzyme with eventually opposite selectivity).

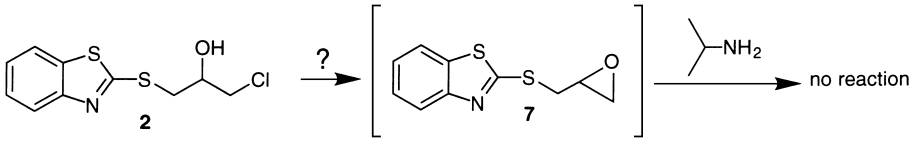
In conclusion, several methods, both chemical (Scheme 4) and chemoenzymatic (Schemes 3 and 5), have been proved to be useful for the synthesis of optically active 1-(benzothiazol-2-ylsulfanyl)-3-chloropropan-2-ol **2** in good to high enantiomeric excess (ee = 70–98%). This compound, as mentioned above, constitutes in turn an interesting chiral synthon C3, a possible precursor of optically active aminoalcohols **6** (with potential β-blocker activity), as well as a starting compound for other different optically active molecules. Concerning the conversion into isopropyl aminoalcohol **6**, for the moment it has been proved feasible (with 30% overall chemical yields) in the case of racemic **2**, following the procedure depicted in Scheme 6.

In this procedure the chlorohydrin **2**, once synthesized by the reduction of **1** or from benzothiazole-2-thiol and epichlorohydrin, was allowed to react with potassium phthalimide giving 1-(benzothiazol-2-ylsulfanyl)-3-phtalimidopropan-2-ol **4**, which in turn by treatment with aqueous hydrazine affords the aminoalcohol **5**. From this the isopropylamino derivative **6** is finally



Scheme 6.

obtained by reaction with $NaBH_4$/acetone. On the other hand, another attempt based on a different approach (treatment of **2** with a base and then with isopropylamine, possibly via the epoxide intermediate **7**: Scheme 7) failed. Similarly, the synthesis starting directly from the racemic epoxide **7** (previously obtained in turn from benzothiazole-2-thiol and racemic epichlorohydrin)[21] with isopropylamine also failed.



Scheme 7.

Pharmacological activity of racemic **6** is under evaluation and will be reported separately.

3. Experimental

3.1. General methods

Melting points taken on Electrothermal apparatus are uncorrected. ^1H NMR spectra were recorded in $CDCl_3$ on a Varian EM 390 or XL 200 spectrometer and chemical shifts are reported in parts per million (δ) from internal Me_4Si. Absolute values of the coupling constant are reported. IR spectra were recorded on a Perkin–Elmer 681 spectrometer. GC analyses were performed by using an HP1 column (methyl silicone gum; 5 m×0.53 mm×2.65 μm film thickness) on an HP 5890 model, Series II. HPLC analyses for the determination of enantiomeric excesses were carried out using a Daicel Chiralcel OD column (tris-3,5-dimethylphenylcarbamate, derivatized cellulose film) on an HP series 1050 instrument. Optical rotations were measured on a Perkin–Elmer digital polarimeter, model 241 MC. Thin-layer chromatography (TLC) was performed on silica gel sheets with a fluorescent indicator (Statocrom SIF, Carlo Erba); the spots on the TLC were

observed under ultraviolet light or were visualized with I_2 vapour. Flash chromatography was conducted by using silica gel with an average particle size of 60 μm, a particle size distribution 40–63 μm and 230–400 ASTM. GC–MS analyses were performed on an HP 5995C model and microanalyses on an Elemental Analyzer 1106-Carlo Erba instrument.

3.2. Materials

The enzymes used were obtained from either the Amano Enzyme Co. or the Sigma Chemical Co. All other chemicals and solvents of the highest quality grade available were purchased from the Aldrich Chemical Co. or the Sigma Chemical Co.

3.3. 1-(Benzothiazol-2-ylsulfanyl)-3-chloropropan-2-one **1**

A suspension of benzothiazole-2-thiol (0.055 mol) and $NaHCO_3$ (0.165 mol) in 360 ml of acetone was stirred at room temperature for 30 min. Then the dichloroacetone (0.110 mol in 240 ml of acetone) was added in a dropwise manner. The reaction mixture was stirred under reflux for a further 6 h, then cooled at 0°C and filtered. The obtained solution was concentrated under vacuum. The residue was treated with ethyl acetate and then washed with 0.1N NaOH. The extracts were dried over Na_2SO_4 and the solvent evaporated. The crude yellowish 1-(benzothiazol-2-ylsulfanyl)-3-chloropropan-2-one obtained was treated with hexane:ethyl acetate (3:1) and stirred at room temperature for 12 h. The precipitated yellow solid was isolated by filtration. The crude product was extracted from the filtrate with hexane for 24 h by using a Soxhlet. The extract was further purified by chromatography (silica gel, petroleum ether:ethyl acetate = 8:2) and then recrystallized from EtOH:H_2O (8:2). The yield of the ketosulfide as white solid was 46%. Mp 101.5–102.5°C (EtOH/H_2O); IR (KBr): 1732, 1450, 1420, 1390, 1380, 1360, 1310, 1275, 1235, 1040, 1000, 755, 720 cm^{-1}; ^1H NMR ($CDCl_3$, δ): 7.82–7.73 (m, 2H, aromatic protons), 7.43–7.27 (m, 2H, aromatic protons), 4.44 (s, 2H, $ClCH_2$), 4.31 (s, 2H, SCH_2). GC–MS (70 eV) m/z (rel. int.): 257 (M^+, 24), 208 (34), 182 (10), 181 (17), 180 (100), 167 (9), 136 (27), 45 (12). Anal. calcd for $C_{10}H_8NOS_2Cl$: C, 46.69; H, 3.11; N, 5.45. Found: C, 46.62; H, 3.01; N, 5.34.

3.4. $NaBH_4$/EtOH reduction of 1-(benzothiazol-2-ylsulfanyl)-3-chloropropan-2-one **1**

To a mixture of ketosulfide **1** (600 mg, 3.88 mmol) in ethyl alcohol (30 ml) kept in an ice-bath was added $NaBH_4$ (100 mg, 4.63 mmol). The reaction mixture was stirred at 0°C for 2 h. Then the ethyl alcohol was evaporated in vacuo. The residue was treated with 30 ml of H_2O and extracted three times with ethyl acetate (30 ml). The extracts were dried over anhydrous Na_2SO_4 and the solvent evaporated. The crude product contained 80 mg of unreacted ketone and 500 mg of product, that were separated by chromatography (silica gel, petroleum ether:ethyl acetate = 8:2) and purified by crystallization (petroleum ether:ethyl acetate = 8:2). The pure chlorohydrin **2** was obtained in 70% yield. Mp 89–91°C; IR (nujol): 3500–3120, 1454, 1425, 1373, 1357, 1312, 1274, 1246, 1231, 1094, 1078, 1027, 1016, 989, 755 cm^{-1}; ^1H NMR ($CDCl_3$, δ): 7.84–7.72 (m, 2H, aromatic protons), 7.45–7.29 (m, 2H, aromatic protons), 4.32–4.25 (m, 1H, -C*H*(OH)-), 3.80–3.58 (bs, 1H, OH: exchange with D_2O), 3.69–3.68 (m, 2H, -CH_2Cl and 1H, -CH_2S-), 3.59–3.52 (dd, J = 6.21 and 14.84 Hz, 1H, -CH_2S-). GC–MS (70 eV) m/z (rel. int.): 259 (M^+, 10), 210 (27), 181 (23), 180 (16), 168 (11), 167 (100), 148 (19), 136 (15), 121 (7), 107 (17), 69 (12), 45 (16). Anal. calcd for $C_{10}H_{10}NOS_2Cl$: C, 46.33; H, 3.86; N, 5.39. Found: C, 46.94; H, 4.10; N, 5.36.

The racemic alcohol was prepared to compare the analytical data of the corresponding product derived from both the reduction of the ketosulfide performed in the presence of the baker's yeast and optically active product derived from the kinetic resolution by lipases, to set the condition for the HPLC analysis to determine the enantiomeric excesses of the optically active alcohol, and to accomplish the synthesis of **6**.

The chromatography separability factor α of (–)- and (+)-alcohol was fair but not suitable for enantiomeric excess determination. On the contrary, α for the acetyl derivative **3** was high enough.

The list of the HPLC retention time (t_R) and the chromatography separability factor (α) found for the (±)-alcohols **3** is as follows:

compound	(R)-(+)-**2**	(S)-(-)-**2**	(R)-(+)-**3**	(S)-(-)-**3**
t_R	20.27	18.57	29.32	19.57
k'	4.2	3.8	6.4	3.9
α	1.10		1.64	

The HPLC analyses were executed in isocratic manner, flow rate = 1.0 ml/min, sparge = 25 ml/min, mobile phase: hexane:*i*-PrOH = 98:2.

3.5. *Synthesis of (R)-(+)- and (S)-(–)-1-(benzothiazol-2-ylsulfanyl)-3-chloropropan-2-ol* **2**

To a solution of benzothiazole-2-thiol (1 g, 6 mmol) in methanol (20 ml) was added Na_2CO_3 (0.95 mg, 9 mmol). After 15 min epichlorohydrin[21] (0.55 mg, 6 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 6 h. Then the solvent was removed under reduced pressure. The residue was solubilized in ethyl acetate and washed with water. The organic layer was dried with anhydrous Na_2SO_4 and concentrated under reduced pressure, and at room temperature yielded a reaction crude that was purified by chromatography (silica gel, petroleum ether:ethyl acetate = 10:1), 90% yield of **2**.

3.5.1. *(R)-(+)-1-(Benzothiazol-2-ylsulfanyl)-3-chloropropan-2-ol* **2**

$[\alpha]_D^{20} = +22.8$ (*c* 1 in $CHCl_3$), ee = 98%; spectroscopic and analytical data were identical to those reported above for the racemic compound. Anal. calcd for $C_{10}H_{10}NOS_2Cl$: C, 46.33; H, 3.86; N, 5.39. Found: C, 46.45; H, 4.03; N, 5.29.

3.5.2. *(S)-(–)-1-(Benzothiazol-2-ylsulfanyl)-3-chloropropan-2-ol* **2**

$[\alpha]_D^{20} = -21.8$ (*c* 1 in $CHCl_3$), ee = 96.4%; spectroscopic and analytical data were identical to those reported above for the racemic compound. Anal. calcd for $C_{10}H_{10}NOS_2Cl$: C, 46.33; H, 3.86; N, 5.39. Found: C, 46.03; H, 4.05; N, 5.40.

3.5.3. *(R)-(+)-1-(Benzothiazol-2-ylsulfanyl)-3-chloropropan-2-acetoxy* **3**

$[\alpha]_D^{20} = +9.6$ (*c* 1 in $CHCl_3$), ee = 98%; spectroscopic and analytical data were identical to those reported below for the racemic compound.

3.5.4. *(S)-(–)-1-(Benzothiazol-2-ylsulfanyl)-3-chloropropan-2-acetoxy* **3**

$[\alpha]_D^{20} = -9.5$ (*c* 1 in $CHCl_3$), ee = 96.4%; spectroscopic and analytical data were identical to those reported below for the racemic compound.

3.6. Baker's yeast-mediated reduction of 1-(benzothiazol-2-ylsulfanyl)-3-chloropropan-2-one **1** in aqueous media

A typical procedure for the reduction of **1** is described below.

Fresh Eridania baker's yeast (12 g) was dispersed to a smooth paste in tap water (230 ml). The heterogeneous mixture, with added glucose or sucrose (5 g) and additive or phosphate buffer as from Table 1, was stirred at 37°C and 250 rpm for 30 min. Then ketosulfide **1** (750 mg) was added in three portions (each aliquot every 30 min). The reaction was followed by TLC and stopped at the indicated time (see Table 1). The mixture was saturated with sodium chloride, filtered on Celite. The cake was washed with methylene chloride and then the solution was extracted with ethyl acetate several times. The combined organic extracts were dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the alcohol **2** was purified or separated from the remaining unreacted substrate **1** by chromatography (silica gel, eluent: petroleum ether:EtOAc = 8:2).

Inhibition experiments were carried out in the presence of 12.5, 62.5, or 100 μl of allyl alcohol, respectively, added just after glucose (Table 1, entry 7).

3.7. Procedure for yeast reduction of **1** in hexane

1-(Benzothiazol-2-ylsulfanyl)-3-chloropropan-2-one **1** (50 mg, 0.2 mmol) was added to a 50 ml round-bottomed flask containing 500 mg of yeast (already stirred at room temperature for 3 h with 10 ml of hexane) and 0.16 ml of water (see Table 3), and stirred at room temperature for 48 h. The mixture was filtered and the yeast washed with ethyl acetate (20 ml). The solvent was then removed under reduced pressure. The product was isolated as described in the previous procedure (Section 3.6).

3.7.1. (R)-(+)-1-(Benzothiazol-2-ylsulfanyl)-3-chloropropan-2-ol **2**

Compound **2**, isolated from the last two reactions, had spectroscopic and analytical data identical to those reported above for the racemic compound.

3.7.2. (+)-1-(Benzothiazol-2-ylsulfanyl)-3-chloro-2-acetoxy propane **3**

A solution of chlorohydrin **2** (30 mg, 0.66 mmol) in pyridine (0.4 ml) and Ac_2O (0.4 ml) was stirred at room temperature for 2 h. The reaction was quenched with H_2O, diluted with ethyl acetate (30 ml), and then washed with 5% HCl (2×10 ml) and sat. aq. $NaHCO_3$ solution. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure; the residue was chromatographed (silica gel, petroleum ether:ethyl acetate, 8:2) affording the product in a quantitative yield. $[\alpha]_D^{20} = +7.1$ (*c* 1 in $CHCl_3$), ee = 58%; oil; IR (neat): 3062, 2957, 1744, 1560, 1463, 1428, 1371, 1309, 1275, 1227, 1126, 1077, 988, 933, 757, 727 cm^{-1}; ^1H NMR ($CDCl_3$, δ): 7.86–7.72 (m, 2H, aromatic protons), 7.43–7.26 (m, 2H, aromatic protons), 5.45–5.38 (m, 1H, -CH(OAc)-), 3.83–3.76 (m, 2H, $-CH_2Cl$ and 1H, $-CH_2S$-), 3.63–3.56 (dd, J = 6.58 and 14.27 Hz, 1H, $-CH_2S$-), 2.06 (s, 3H, CH_3COO). GC–MS (70 eV) *m*/*z* (rel. int.): 301 (M^+, 25), 206 (19), 193 (12), 192 (100), 173 (9), 167 (63), 135 (19), 108 (17), 45 (11), 43 (94).

3.8. Kinetic resolution of (±)-chlorohydrin **2**

Crude lipase (100 mg, or 500 mg as from Table 3) and vinyl acetate (1 ml) were added to a solution of racemic alcohol (100 mg) in 10 ml hexane. The heterogeneous mixture was incubated

at 37°C and stirred at 250 rpm. The reaction was followed by TLC and stopped at the indicated time (Table 3). The mixture was filtered through a sintered glass funnel to recover the enzyme extract. The hexane was removed under reduced pressure. The product and remaining unreacted substrate were separated by chromatography (silica gel, eluent: petroleum ether:ethyl acetate = 8:2).

The enantiomeric excesses were determined by chiral HPLC on a column supplied from Diacel (Chiralcel OD) as well as on the mixture containing the remaining substrate and product, just after the filtration. The extent of the conversion was also determined by the same HPLC chromatogram. All these data allow the calculation of the enantiomeric excess of the remaining alcohol and the enantioselective factor value E as well (Table 3).

3.9. Synthesis of 1-(benzothiazol-2-ylsulfanyl)-3-phthalimidopropan-2-ol **4**

A mixture of 1-(benzothiazol-2-ylsulfanyl)-3-chloropropan-2-ol (1.35 g, 5.2 mmol), *N*-potassium phthalimide (1.06 g, 5.7 mmol), and NaI (0.78 g, 5.2 mmol) in 12 ml of DMF kept under nitrogen atmosphere was heated at 80°C for 5 h. Then the precipitate was filtered off and the solution was evaporated to dryness in vacuo. The residue was solubilized by ethyl acetate and washed twice with H_2O. The organic extract was dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the phthalimido derivative was purified by chromatography (silica gel, eluent: petroleum ether:ethyl acetate = 7:3). The product (637 mg) was isolated as white crystals (47% yield). Mp 126–127°C; IR (KBr): 1770, 1700, 1460, 1430, 1395, 1365, 1320, 1085, 1010, 845, 755, 720 cm^{-1}; ^1H NMR ($CDCl_3$, δ): 7.88–7.69 (m, 6H, aromatic protons), 7.40–7.28 (m, 2H, aromatic protons), 4.43–4.36 (m, 1H, -C*H*(OH)-), 4.02–3.95 (dd, J = 6.85 and 14.05 Hz, 1H, CH_2N), 3.92–3.85 (dd, J = 5.40 and 14.05 Hz, 1H, CH_2N), 3.61–3.55 (dd, J = 3.32 and 14.76 Hz, 1H, -CH_2-S-), 3.46–3.39 (dd, J = 6.38 and 14.64 Hz, 1H, -CH_2-S-), 3.50–2.65 (bs, 1H, OH: exchange with D_2O). GC–MS (70 eV) m/z (rel. int.): 370 (M^+, 2), 211 (10), 210 (100), 181 (14), 167 (18), 160 (23), 148 (12), 77 (13). Anal. calcd for $C_{18}H_{14}N_2O_3S_2$: C, 58.37; H, 3.78; N, 7.56. Found: C, 57.99; H, 3.85; N, 7.38.

3.10. Synthesis of 1-(benzothiazol-2-ylsulfanyl)-3-aminopropan-2-ol **5**

To a solution of 1-(benzothiazol-2-ylsulfanyl)-3-phthalimidopropan-2-ol (0.5 g, 1.35 mmol) in 4 ml of methyl alcohol was added AcOH (0.25 ml, 4.08 mmol) and then 55% aq. N_2H_4 (0.25 ml, 4.08 mmol). The reaction mixture was stirred at room temperature for 3 h. A white solid started precipitating after the first 30 min. At the end of the reaction the mixture was filtered. The solvent was evaporated in vacuo and the residue was treated with ethyl acetate and then with 2N HCl. To the aqueous extract 2N NaOH was added to pH > 7, then extracted with ethyl acetate. A yellow oil was obtained by evaporating the solvent, 90% yield. Oil; IR (nujol): 3500–3120, 1454, 1425, 1379, 1357, 1312, 1274, 1246, 1231, 1094, 1078, 1027, 1016, 989, 755 cm^{-1}; ^1H NMR ($CDCl_3$, δ): 7.79–7.76 (m, 2H, aromatic protons), 7.37–7.26 (m, 2H, aromatic protons), 3.96–3.88 (m, 1H, -C*H*(OH)-), 3.49–3.43 (dd, J = 4.40 and 14.05 Hz, 1H, -CH_2-S-), 3.38–3.31 (dd, J = 6.75 and 14.05 Hz, 1H, -CH_2-S-), 3.30–2.90 (bs, 3H, NH_2 and OH: exchange with D_2O), 2.89–2.83 (dd, J = 4.20 and 12.80 Hz, 1H, -CH_2-N), 2.79–2.73 (dd, J = 7.01 and 12.80 Hz, 1H, -CH_2-N). GC–MS (70 eV) m/z (rel. int.): 210 (M^+–30, 100), 168 (18), 167 (93), 108 (10). Anal. calcd for $C_{10}H_{12}N_2OS_2$: C, 49.98; H, 5.03; N, 11.67. Found: C, 50.10; H, 5.29; N, 11.60.

3.11. Synthesis of 1-(benzothiazol-2-ylsulfanyl)-3-N-isopropylaminopropan-2-ol **6**

1-(Benzothiazol-2-ylsulfanyl)-3-aminopropan-2-ol **5** (0.29 g, 1.22 mmol) was dissolved in 10 ml of absolute ethanol and 0.7 ml of acetone. Then $NaBH_4$ (0.23 g, 6.1 mmol) was slowly added. After 5 min 4 ml of acetone were added in three portions and the reaction mixture stirred at room temperature for 1 h. Hence, pH was lowered to 5 by 6N HCl, and the ethanol evaporated. The residue was partitioned between aqueous 5% NaOH and ethyl ether. The ether layer was washed with 5% NaOH (3×10 ml), dried with anhydrous Na_2SO_4 and evaporated, affording an oil (223 mg, 65% yield). IR ($CHCl_3$): 3500–3400, 2994, 1444, 1412, 1364, 1357, 1312, 1087 cm^{-1}; ^1H NMR ($CDCl_3$, δ): 7.82–7.70 (m, 2H, aromatic protons), 7.41–7.24 (m, 2H, aromatic protons), 4.13–3.99 (m, 1H, -C*H*(OH)-), 3.57–3.51 (dd, J = 4.40 and 14.15 Hz, 1H, -CH_2S-), 3.46–3.41 (dd, J = 6.60 and 14.15 Hz, 1H, -CH_2S-), 3.20–2.60 (bs, 2H, NH and OH: exchange with D_2O), 2.85–2.79 (dd, J = 4.40 and 12.10 Hz, 1H, -CH_2N), 2.85–2.73 (m, 1H, CH/*i*-Pr, this signal is overlapped with the previous one; nevertheless, the two systems can be clearly identified), 2.73–2.67 (dd, J = 7.30 and 12.10 Hz, 1H, -CH_2N), 1.06–1.03 (2d, J = 2.05 and 6.18 Hz, 6H, 2 CH_3/*i*-Pr). GC–MS (70 eV) *m*/*z* (rel. int.): 282 (M^+, 1), 209 (20), 167 (16), 122 (3), 98 (19), 72 (100), 43 (11). Anal. calcd for $C_{13}H_{18}N_2OS_2$: C, 55.32; H, 6.38; N, 9.93. Found: C, 55.10; H, 6.29; N, 9.60.

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