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Lipase Mediated Chiral Resolution of 4-Arylthio-2-Butanol as an Intermediate for β-Lactam Antibiotics

Kwang-Jin Hwang, Jinkue Lee, Sungmin Chin, Chi Jang Moon[1], Wonjae Lee[2], Chae-Sun Baek[2], and Hyung Jin Kim[3]

Department of Chemical System Engineering, Hongik University, Jochiwon, Chungnam 339-701, Korea, [1]Choongwae Pharma Corporation, Taean-eup Hwasung-kun Kyungki-do, Korea, [2]College of Pharmacy, Chosun University, Kwangju, Korea, and [3]Department of Applied Chemistry, Chonnam National University, Kwangju, 500-757, Korea

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This paper deals with chiral enzymatic resolution of 4-arylthio-2-butanols by lipase to prepare potential intermediates of β-lactam antibiotics. Among several lipases employed, lipase P type enzyme gave the highest *ee* value to prepare (*R*)-4-arylthio-2-butyl acetate. The enzymatic resolution of phenyl substituted alcohol (**6a**) using lipase P showed the highest ee value (99.7%) among those of 4-arylthio-2-butanol derivatives. Lipase P mediated hydrolysis of acylester **7a** gave also (*R*)-alcohol **6a** selectively. For determination of enantiomeric purity of these enzymatic resolved analytes, liquid chromatographic analysis was performed using two coupled Chiralcel OD and (*R,R*)-WhelkO chiral column.

Key words: Lipase, 4-Arylthio-2-butanol, Chiral resolution

INTRODUCTION

Optically active phenylthioazetidin-2-one (**1**) is an essential intermediate for the preparation of penem and carbapenem antibiotics (Berks, 1996). Azetidinone (**1**) is conveniently prepared by the [2+2] cycloaddition of the alkenol **2** with chlorosulfonyl isocyanate and then transformed to the next intermediate for β-lactams *via* dethioaroylation as reported by Ishiguro and coworkers (Shimamoto, 1994; Nakatsuka, 1991). In the synthesis of azetidinone (**1**), the absolute configuration of 1-hydroxyethyl group originates from that of alkenol (**2**). Thus, the enzymatic resolution of alkenol (**2**) garnered industrial interest by many groups (Sih, 1992; Yamashita, 1996). Since a protected alcohol, (*R*)-4-phenylthio-2-silyloxybutane (**3**) is readily converted to the alkenol (**2**) by the halogenation and subsequent dehalogenation reactions as key steps (Nakatsuka, 1991), optically active 4-arylthio-2-butanol derivative (**6**) has been considered as a useful precursor for the β-lactam antibiotics. An enzymatic resolution of phenylthio-2-butanol (**6a**) from the corresponding ketone (**5a**) was reported (Ohtsuka, 1995; Sugai, 1996) using a reductase *Pichia farinosa*. The enzyme, however, used in the reduction is cultured from their own microorganism limiting its applications in general.

In an effort to provide an efficient way for chiral resolution of arylthiobutanol (**6**), we employed the lipase mediated transesterification using vinylaceate, a representative enzymatic resolving method of secondary alcohol (Wang, 1988). Several 4-arylthio-2-buthanol derivatives (**6a-e**) with various substituents at the phenyl ring were prepared in consideration of the substituent effect on the enzyme substrate selectivity in the enzymatic chiral resolution as well as the reactivity control in removing arylthio group for the conversion to the alkenol (**2**) (Nakatsuka, 1991). Here, we report the preparation and lipase mediated resolution of the 4-arylthio-2-butanol derivatives (**6a-e**).

MATERIALS AND METHODS

Chromatographic analysis was performed at room temperature using an HPLC consisting of a Waters model 510 pump, a Rheodyne model 7125 injector with 20 mL loop, a variable wavelength detector (Dynamax UV-1, Rainin), and a Waters 746 data module integrating recorder. Directions of optical rotation were determined using a Shodex OR-1M (Showa Denko, Japan) as an inline chroma-

Correspondence to: Kwang-Jin Hwang, Department of Chemical System Engineering, Hongik University, Jochiwon, Chungnam 339-701, Korea
Tel: 82-41-860-2217, Fax: 82-41-866-6940
E-mail: kjhwang@wow.hongik.ac.kr

tography detector. Chiralcel OD and Chiralcel OJ purchased from Daicel Chemical Company (250 mm L×4.6 mm I.D., Tokyo, Japan) and *(R,R)*-WhelkO purchased from Regis Technologies (250 mm L×4.6 mm I.D., USA) were used to determine enantiomeric purity of analytes. The analytes were detected by UV at 254 nm. Lipases were purchased from the Amano or Fluka company.

Synthesis of optically active alcohol (*R*)-6a

A mixture of methyl (*R*)-3-hydroxybutanoate (1 equiv), imidazole (2.5 equiv) and *tert*-butyldimethylsilyl chloride (1.5 equiv) in DMF was stirred for 12 h at room temperature. The solution was diluted with water and extracted twice with Et_2O, and the organic extracts were dried ($MgSO_4$) and evaporated. Column chromatograph on silica (5% EtOAc/*n*-hexane) gave methyl (*R*)-3-(*tert*-butyldimethylsilanyloxy) butyrate (95%). An etheral solution of this ester was slowly added to a suspension of $LiAlH_4$ (1.5 equiv) in dry Et_2O and the mixture was refluxed for 6 h. The mixture was cooled to 0°C then water was added. The organic layer separated, washed with brine, dried ($MgSO_4$) and concentrated to give (*R*)-3-(*tert*-butyldimethylsilanyloxy) butan-1-ol, which was dissolved in Et_2O, then pyridine (1.2 equiv) and tosyl chloride (1.2 equiv) was added at 0°C. After stirring for 12 h, the reaction mixture was successively washed with water, 10% HCl solution, sat'd $NaHCO_3$ solution and brine. The resulting solution was dried ($MgSO_4$) and concentrated to give the crude (*R*)-3-(*tert*-butyldimethylsilanyloxy)butyl toluenesulfonate (80%). A mixture of this tosylate and sodium phenylthiolate (1.2 equiv) in THF was stirred at room temperature for 24 h to afford (*R*)-3-(*tert*-butyldimethylsilanyloxy)butyl phenylsulfide, which was treated with a THF solution of tetrabutylammonium fluoride (5 equiv) to give the crude (*R*)-**6a.** The purification using column chromatography (20% EtOAc/*n*-hexane) gave a clear liquid: $[\alpha]_D^{20}$ = -20.1 (c=3.6) in $CDCl_3$; ^{1}H-NMR (200 MHz) δ 7.40-7.32 (5H, m, ArH), 3.95 (1H, q, 6 Hz, CH), 3.03 (2H, m, CH_2SPh), 2.53 (1H, s, OH), 1.76 (2H, m, CH_2), 1.20 (3H, d, 6 Hz, CH_3).

Lipase mediated acylation of alcohol 6

A suspension of racemic 4-phenylsulfanyl-butan-2-ol (**6a**, 207 mg, 1.13 mmol), vinyl acetate (120 mg, 1.40 mmol) and lipase P (20.1 mg) in *n*-hexane (1 mL) was stirred at room temperature until the alcohol was converted to the ester in about 50% yield by GC analysis. The reaction mixture was diluted by adding ethyl acetate and then the lipase was filtered off through celite. The organic layer was concentrated under reduced pressure and loaded on the column chromatography (10% EtOAc/*n*-hexane) to give the ester **7a** (109 mg, 43%) with unreacted alcohol (93 mg, 45%). The acylations of the other arylthio-2-butanols (**6b-e**) with various lipases were proceeded under the similar reaction condition to that in case of alcohol **6a**.

Hydrolysis of acylester 7a

A solution of 4-phenylsulfanyl-2-butyl acetate (**7a**, 158 mg, 0.71 mmol) in a mixture of H_2O (1 mL) and *n*-hexane (1 mL) was suspended with lipase P (23 mg) at 30-40°C and stirred for 20 h. When the reaction proceeded with approximately 50% conversion, ethyl acetate was added to the reaction mixture then the resulting mixture was filtered through celite. After washed with brine the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (20% EtOAc/*n*-hexane) to afford the alcohol **6a** (37 mg, 28%) and unreacted ester (114 mg, 72%).

Synthesis of ketones 5a-e

To a solution of methyl vinyl ketone (202 mg, 2.88 mmol) in THF (3 mL) was added aqueous 10% NaOH solution (3 mL) and thiophenol (322 mg, 2.92 mmol) then the mixture was stirred overnight at 70-80°C. After addition of ethyl acetate to the reaction mixture, the organic layer was washed with brine and dried over $MgSO_4$ then concentrated. The residue was purified by column chromatography (20% EtOAc/*n*-hexane) to give butanone **5a** (106 mg, 20%); ^{1}H-NMR (200 MHz) δ 7.36-7.21 (5H, m ArH), 3.14 (2H, t, 7.4 Hz, SCH_2), 2.76 (2H, t, 7 Hz, CH_2), 2.15 (3H, s, CH_3). By the similar method to **5a**, other ketones **5b-e** were prepared: **5b** (60%), 7.40-7.05 (4H, m, ArH), 3.15 (2H, t, 7.2 Hz, SCH_2), 2.79 (2H, t, 7.4 Hz, CH_2), 2.18 (3H, s, CH_3); **5c** (25%), 7.23 (4H, s, ArH), 3.08 (2H, t, 7.4 Hz, SCH_2), 2.72 (2H, t, 7.2 Hz, CH_2), 2.11 (3H, s, CH_3); **5d** (44%), 7.25-6.91 (3H, m, ArH), 3.05 (2H, t, 7.4 Hz, SCH_2), 2.72 (2H, t, 7.4 Hz, CH_2), 2.35 (6H, s, $ArCH_3$), 2.12 (3H, s, CH_3), **5e** (57%), 7.31-6.77 (4H, m, ArH), 3.73 (3H, s, OCH_3), 2.96 (2H, t, 7.2 Hz, SCH_2), 2.63 (2H, t, 7.2 Hz, CH_2), 2.10 (3H, s, CH_3).

Reduction of ketone 5 to alcohol 6

To a solution of the butanone (**5a**, 592 mg, 3.26 mmol) in THF (5 mL) was added $NaBH_4$ (54 mg, 1.42 mmol) then the mixture was stirred overnight at 75°C. Ethyl acetate and brine were added to the mixture. The organic layer separated, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography (20% EtOAc/*n*-hexane) to give alcohol **6a** (334 mg, 56%); ^{1}H-NMR (200 MHz) δ 7.36-7.21 (5H, m, ArH), 4.0 (1H, q, 6 Hz, CH), 3.05 (2H, m, SCH_2), 1.76 (2H, q, 7 Hz, CH_2), 1.58 (1H, s, OH), 1.23 (3H, d, 6.2 Hz, CH_3). Other alcohols **6b-e** were prepared by the similar procedures as in case of alcohol **6a** and their yields and ^{1}H-NMR data are summarized as follow: **6b** (67%), δ 7.34-7.21 (4H, m, ArH), 4.0 (1H, q, 6 Hz, CH), 3.04 (2H, m, SCH_2), 1.79 (2H, q, 7.4 Hz, CH_2), 1.24 (3H, d, 6.2 Hz,

CH_3); **6c** (58%), 7.22 (4H, s, ArH), 3.90 (1H, q, 6 Hz, CH), 2.96 (2H, m, SCH_2), 2.33 (1H, s, OH), 1.69 (2H, q, 6 Hz, CH_2), 1.18 (3H, d, 6.4 Hz, CH_3); **6d** (75%), δ 7.23-7.00 (3H, dd, 7.6 Hz, ArH), 3.90 (1H, q, 6 Hz, CH), 2.94 (2H, m, SCH_2), 2.35 (3H, s, $ArCH_3$), 2.24 (3H, s, $ArCH_3$), 1.95 (1H, s, OH), 1.77 (2H, q, 6 Hz, CH_2), 1.18 (3H, d, 6.4 Hz, CH_3); **6e** (84%), δ 7.36-6.81 (4H, m, ArH), 3.90 (1H, q, 7.2 Hz, CH), 3.77 (3H, s, OCH_3), 2.91 (2H, m, SCH_2), 1.95 (1H, s, OH), 1.71 (2H, q, 7.4 Hz, CH_2), 1.18 (2H, d, 6.4 Hz, CH_3).

1-Methyl-3-(phenylsulfanyl)propyl acetate (7a)

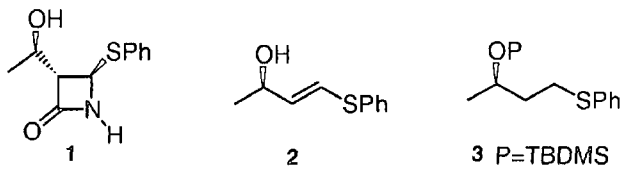
To a solution of the butanol (**6a**, 513 mg, 2.81 mmol) in CH_2Cl_2 (2 mL) was added pyridine (0.6 mL) and acetic anhydride (1.59 g, 15.54 mmol) and then the resulting mixture was stirred overnight at room temperature. Ethyl acetate and water were added to the reaction mixture, then the organic layer was separated, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified using column chromatography (20% EtOAc/*n*-hexane) to give the acetate **7a** (532 mg, 85%).

RESULTS AND DISCUSSION

The alcohols **6a-e** were prepared by the addition reaction of arylthiol (**4**) to methyl vinyl ketone (Liu, 1995) followed by $NaBH_4$ reduction, as shown in the Scheme 1. The standard alcohol (*R*)-**6a** was prepared from (*R*)-methyl 3-hydroxybutanoate by the sequence of reactions (TBDMS protection, LAH reduction and tosylation) followed by phenylthiolate displacement and deprotection of alcohol group, as indicated in Scheme 2.

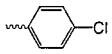
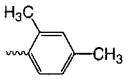
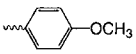
Several lipases (P, PS, type II, type VII) were initially tested for the acylation reaction of racemic 2-butanol (**6a**) with vinyl acetate. Among the tested lipases, only lipase P gave considerable *ee* values (>70%) while the enzymatic resolutions using other lipases resulted in low *ee* values (5-10%). The results implies that the acylation of the alcohols (**6a-e**) as well as the selective hydrolysis of the acetate (**7**) can be mediated efficiently by lipase P. The results for the acylation reactons are summerized in Table I.

For determinination of enantiomeric purity of enzymatically resolved 4-arylthio-2-butanol compounds (**6a-e**), liquid chromatographic analyses were performed using several chiral columns, such as Chiralcel OJ, Chiralcel OD and (*R,R*)-WhelkO. The elution orders of enantiomers (**6a**) on these chiral HPLC columns were determined by injecting the standard sample *(R)*-**6a**, prepared in Scheme 2. The directions of optical rotation of the enantiomers of racemic **6a** were determined with an in-line polarimetric detector set at 589 nm. However, Chiralcel OJ, Chiralcel OD and (*R,R*)-WhelkO chiral columns did not provide base-line separation of enantiomers (**6a**) (Fig. 1). Since both Chiralcel OD and *(R,R)*-WhelkO columns showed the same elution order for racemic compound (**6a**) as shown in Table II, two coupled Chiralcel OD and *(R,R)*-WhelkO

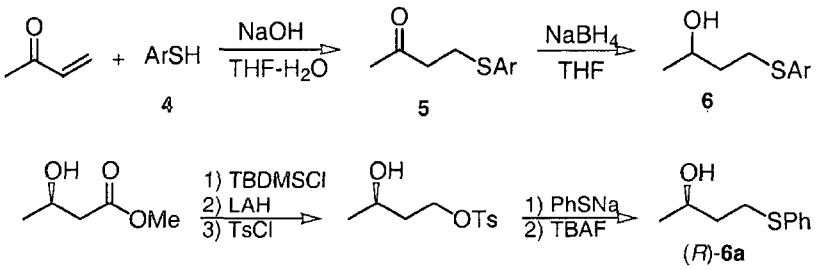


Scheme 1. Molecular structures of Key intermediates

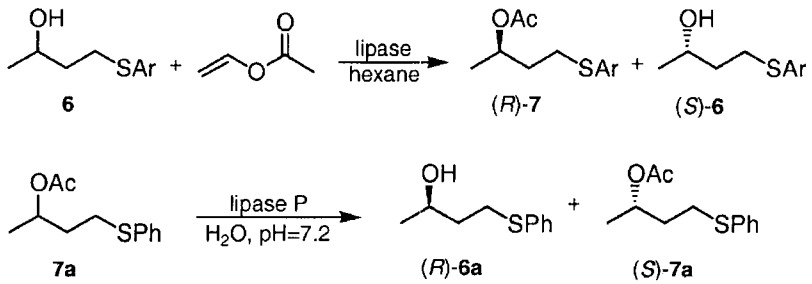
Table I. Lipase P mediated resolution of 4-arylthio-2-butanols (**6**)

alcohol (6)	Ar	% *ee* of (*R*)-**7**[a]	Yield (%)[b]
a		99.7	43
b		90.2	46
c		83.6	43
d		89.8	35
e		89.2	45

[a] The enantiomertic purity was determined on chiral columns by HPLC. [b] Isolated yield after column chromatography.



Scheme 2. Synthetic pathways for alcohols **6** and (*R*)-**6a**



Scheme 3. Enzymatic resolutions of alcohol **6** and ester **7a**

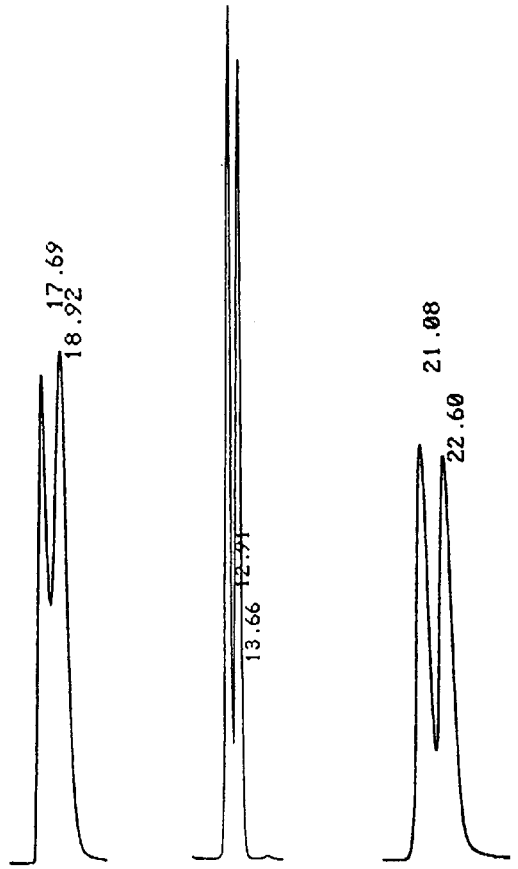


Fig. 1. Chromatograms of enantiomer separation of racemic **6a** on Chiral OJ, Chiralcel OD and *(R,R)*-WhelkO, respectively. See Table II for chromatographic conditions.

Table II. Enantiomer resolution of 4-phenylthio-2-butanol on several chiral HPLC columns

Chiral column		α	k'_1	*Conf.*[a]
1	Chiralcel OJ		1.08	4.90[b] (-)-*R*
2	Chiralcel OD		1.08	3.30[c] (+)-*S*
3	*(R,R)*-WhelkO		1.08	6.03[d] (+)-*S*
4	Chiralcel OD + *(R,R)*-WhelkO		1.10	2.78[b] (+)-*S*

[a] The configuration of the second eluted isomer. [b] Mobile phase = 3.5% 2-propanol in *n*-hexane (*v/v*); flow rate = 1 mL/min; UV 254 nm. [c] 2% 2-Propanol in *n*-hexane (*v/v*) as a mobile phase. [d] 1% 2-Propanol in *n*-hexane (*v/v*) *n*-hexane (*v/v*) as a mobile phase.

was used for improving resolution of enantiomers (**6a**) to result in almost base-line separation. The chromatograms of enantiomer separation of **6a** and determination of the enantiomeric purity of an enantiomerically enriched **6a** on two coupled Chiralcel OD and *(R,R)*-WhelkO are shown in Fig. 2. These coupled columns were also used to determine the enantiomeric purity of other samples in this study. On the other hand, the base-line resolution of the enantiomers **7a** was achieved on Chiralcel OD column.

Enzymatic acylation of **6a** was proceeded in 99.7% *ee*

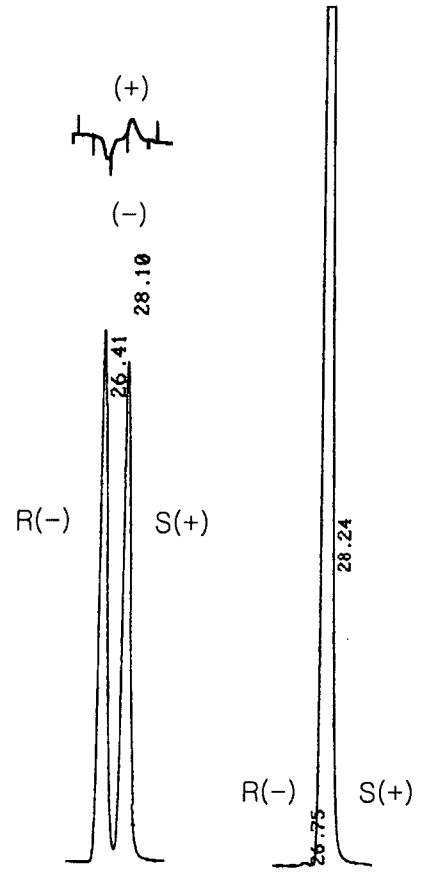


Fig. 2. Chromatograms of enantiomer separation of racemic **6a** and enantiomerically enriched **6a** on two coupled Chiralcel OD and *(R,R)*-WhelkO. See Table II for chromatographic conditions. Directions of optical rotation were determined using a polarimetric detector.

at 43% conversion. The resolution efficacy of 4-arylthio-2-butanols (**6**) using lipase P was varied in the range of 84-90% *ee* depending upon the substituent on aromatic ring of the alcohols (**6**), as shown in Table I. In the case of **6a** substituted with phenyl group, the highest *ee* value was obtained. The lipase mediated chiral resolution of other 4-arylthio-2-butanols (**6b-e**) possessing Me, OMe or Cl substituents at various positions of the phenyl ring gave moderate enantioselectivities (84-90% *ee*). In addition, hydrolysis of acetate **7a** in phosphate buffer solution (pH= 7.2) gave alcohol *(R)*-**6a** with 97% *ee* (47% conversion determined by GC).

In conclusion, the lipase P mediated acylation of alcohols (**6a-e**) gave enantiomerically pure *(R)*-acetate (**7a-e**), a potential intermediate of β-lactam antibiotics, in high *ee* value. Meanwhile, the lipase P mediated hydrolysis of acyl ester (**7a**) generated acetate (*S*)-**7a** along with alcohol (*R*)-**6a** providing a method for complimentary preparation of optically pure enantiomers. The change of substituents at the phenyl ring of alcohol **6** resulted in the resolution with 84-90% *ee* value. Eventually, butanol **6a** with a phenyl group showed the highest *ee* value (99.7%). To determine enantiomeric purity of these alcohols **6a-e,** liquid chromatographic analysis was performed using two coupled Chiralcel OD and *(R,R)*-WhelkO chiral column.

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REFERENCES

Berks, A. H., Preparations of two pivotal intermediates for the synthesis of 1-β-methyl carbapenem antibiotics, *Tetrahedron*, 52, 331-375 (1996).

Kim, B.-H. and Lee, W., Liquid chromatographic resolution of pyrethroic acids and their esters on chiral stationary phases. *J. High Resol. Chromato.*, 21, 189-192 (1998).

Lee, W., Separation of the enantiomers of amino acid esters as benzophenone imine derivatives using polysaccharide derived chiral stationary phases, *Chromatographia*, 49, 61-64 (1999).

Liu., H. and Cohen, T. (*S*)-(+)-5-(Phenylthio)-2-pentanol and (*S*)-(+)-4-(phenylthio)-2-butanol: Readily prepared, useful additions to the chirality pool. highly enantioselective syntheses of naturally occurring spiroketal pheromones. *J. Org. Chem.*, 60, 2022-2025 (1995).

Nakatsuka, T., Iwata, H., Tanaka, R., Imajo, S., and Ishiguro, M., A facile conversion of the phenylthio group to acetoxy by copper reagents for a practical synthesis of 4-acetoxyazetidin-2-one derivatives from (*R*)-butane-1,3-diol. *J. Chem. Soc., Chem. Commun.*, 35, 662-664 (1991).

Ohtsuka, Y., Katoh, O., Sugai, T., and Ohta, H., Preparation of sulfur-containing optically active secondary alcohols based on *Pichia farinosa*-catalyzed anti-Prelog-rule reduction as the key step, *Bull. Chem. Soc. Jpn.*, 70, 483-491 (1997).

Shimamoto, T., Inoue, H., Yoshida, T., Tanaka, R., Nakatsuka, T., and Ishiguro, M., Copper-assisted substitution reaction for phenylthio group of a 4-phenylthioazetidinone derivative, *Tetrahedron Lett.*, 35, 5887-5888 (1994).

Sih, C. J., Process for preparing optically active α-hydroxyalkene derivatives from racemic esters. EP 471531 (1992). *Chem. Abstr.*, 117, 24775 (1992).

Sugai, T., Ohtsuka, Y., and Ohta, H., Biocatalytic preparation of (*R*)-(-)-4-(phenylthio)-2-butanol and (*R*)-(-)-4-(phenylsulfonyl)-2-butanol by the sequential use of *Pichia farinosa* and *Rhodococcus rhodochrous*. *Chem. Lett.*, 233-234 (1996).

Wang, Y.-F., lalonde, J. J., Momongan, M., bergbreiter, D. E., and Wong, C.-H., Lipase-catalyzed irreversible transesterifications using enol esters as acylating reagents: preparative enantio- and regioselective synthesis of alcohols, glycerol derivatives, sugars and organometallics. *J. Am. Chem. Soc.*, 7200-7205 (1988).

Yamashita, K., Kawada, N., Hamaya, T., and Ueda, Y., Microbial reduction of 3-oxo-1-phenylthio-1-butene. JP 08196287. *Chem. Abstr.*, 125, 245827 (1996).