FULL PAPER

Applying Lipase Catalysis to Access the Enantiomers of Dorzolamide Intermediates

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The kinetic resolution of three dorzolamide intermediates has been studied in the presence of *Burkholderia cepacia* lipase in organic solvents. All the stereoisomers of 6-methyl-5,6-dihydro-4*H*-thieno[2,3-*b*]thiopyran-4-ol were prepared starting from the racemic *cis*-dihydrothiopyranol intermediate giving first the 4*R*,6*S* and 4*S*,6*R* enantiomers. Subsequent epimerization and purification of the *trans* enantiomers by enzymatic acylation or alcoholysis then gave the *cis* enantio-

mers. The *cis*-4-hydroxy-6-methyl-5,6-dihydro-4*H*-thieno-[2,3-*b*]thiopyran 7,7-dioxide enantiomers were also prepared by enzymatic kinetic resolution. The kinetic resolution of ethyl 3-(2-thienylthio)butanoate with lipases gave moderate enantioselectivities but the method was not used on a preparative scale with this substrate.
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Introduction

(4*S*,6*S*)-2-(Aminosulfonyl)-4-(ethylamino)-5,6-dihydro-6-methyl-4*H*-thieno[2,3-*b*]thiopyran 7,7-dioxide, also known as dorzolamide (Scheme 1), is a topically active human carbonic anhydrase II inhibitor that affects the hydration of carbon dioxide ($CO_2 + H_2O = HCO_3^- + H^+$) by slowing the formation of hydrogen carbonate with a subsequent reduction in sodium and fluid transport.[1,2] Dorzolamide, as a water-soluble hydrochloride, was formulated to be used in ophthalmic solutions (Trusopt) to reduce elevated intraocular pressure in open-angle glaucoma and ocular hypertension. The first synthesis of the desired 4*S*,6*S* stereoisomer was successfully achieved by the Merck laboratories.[3] In the synthesis, the *S* configuration at C-6 was accomplished by the tosylation of methyl (*R*)-3-hydroxybutanoate obtained from a bacterial poly[(*R*)-3-hydroxybutanoate] followed by S_N2 displacement with 2-(lithiomercapto)thiophene to form (*R*)-**2**.[4–6] The desired stereochemistry at C-4 was introduced in a later step in the synthesis: First, diastereomeric control gave (4*R*,6*S*)-**3** in the reduction of the preceding (6*S*)-4-keto sulfone intermediate and then acid-catalyzed epimerization at C-4 resulted in (4*S*,6*S*)-**3**. Later, a whole-cell biotransformation by the fungus *Neurospora crassa* was developed to replace the two-step protocol to yield (4*S*,6*S*)-**3** directly from the 4-keto sulfone in a single step.[4] In other synthetic routes to dorzolamide, (4*R*,6*S*)-

5,6-dihydro-4-hydroxy-6-methyl-4*H*-thieno[2,3-*b*]thiopyran 7,7-dioxide [(4*R*,6*S*)-**4**] or its *trans* isomer (4*S*,6*S*)-**4** were reported as key intermediates in the synthetic protocol.[7,8] These chiral hydroxy sulfone intermediates were obtained by enzymatic or chemical reduction of the corresponding 4-keto sulfone or by the cyclization of the chiral thienyl-thiobutanoic acid obtained as described above.[9] The importance of chiral key intermediates in various dorzolamide syntheses[4–9] led us to consider the intermediates *rac*-**1**–**4**, which can all function as lipase substrates (Scheme 1). The intermediates can be grouped into two categories: *rac*-**1** and *rac*-**2** may be used to fix the asymmetric center at C-6 of dorzolamide whereas the dihydrothiopyranol derivatives *rac*-*cis*-**3** and *rac*-*cis*-**4** produce the 4*R*,6*S* and 4*S*,6*R* enantiomers. Lipase-catalyzed access to the enantiomers of *rac*-**1** was previously achieved by catalysis with *Candida antarctica* lipase B (Novozym 435).[10] The kinetic resolution of *rac*-**2**, *rac*-*cis*-**3**, and *rac*-*cis*-**4** is now considered. In a broader context, we have focused on the possibility of preparing the four stereoisomers of **3** and also studied the lipase-catalyzed acylation of *rac*-*trans*-**3**. Lipases (EC 3.1.1.3) are especially attractive biocatalysts for synthetic purposes due to their usually broad substrate specificity, high selectivity, effective catalysis without cofactors, and good commercial availability. Highly enantioselective lipase-catalyzed kinetic resolution affords both enantiomers of a racemic mixture simultaneously (one as an unreacted enantiomer and the other as a new reaction product). Thus, the acylation of a racemic secondary alcohol and the alcoholysis of the corresponding racemic ester can produce a resolved mixture in which one of the enantiomers is an alcohol and the other an ester. Inversion of the configuration at the free alcoholic HO group at the asymmetric center can then be used to transform the resolved mixture into an ester enantio-

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mer. The Mitsunobu esterification was first introduced to the lipase-catalyzed kinetic resolution of various secondary alcohols to transform the unreacted alcohol enantiomer into the reacted ester enantiomer.[11] Later, the development of a lipase-catalyzed solvent-free process combined with mesylation and subsequent acylation with caesium acetate allowed an easy and clean access to the acylated ethyl (*R*)-3-hydroxybutanoate from *rac*-**1**.[10] In addition, dynamic kinetic resolution methods, which allow the transformation of a racemic mixture into the more reactive enantiomer, have been widely investigated.[12] When an enantiopure alcohol contains two or more asymmetric centers the resolution methods can lead to a mixture of enantiopure diastereomers.
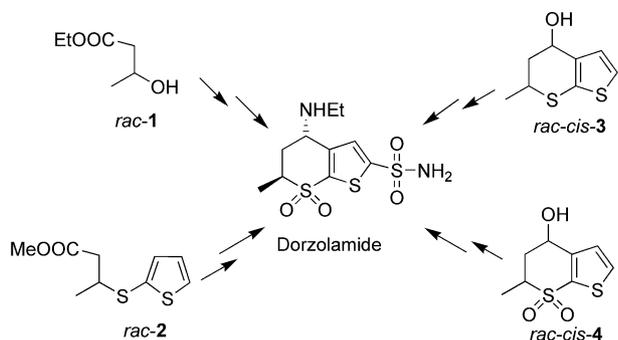

Scheme 1. Potential intermediates of dorzolamide for kinetic resolution.

Results and Discussion

One of the difficulties to be overcome in the original Merck synthesis was the elimination of water from methyl (*R*)-3-hydroxybutanoate and the reaction of the methyl crotonate thus obtained with 2-mercaptothiophene by Michael addition to form *rac*-**2** rather than (*R*)-**2**.[3] We screened *rac*-**2** as a potential substrate for lipase-catalyzed kinetic resolution against 22 commercial lipases from different sources in free and immobilized forms (the reacting ones are shown in the Exptl. Sect.) by using alcoholysis, interesterification, aminolysis, and hydrolysis reactions. With every reaction type and for all the lipases except CAL-B, the same enantiomer of the racemate was the more reactive, as shown by the HPLC chromatograms. The reactive enantiomer was expected to be the *S* stereoisomer on the basis of our previous work[13] with similar substrates (see the aminolysis of *rac*-**2**, Scheme 2). Alcoholysis with *n*-butanol in toluene, diisopropyl ether (DIPE), or *tert*-butyl methyl ether (TBME), interesterification with butyl butanoate in mixtures of butyl butanoate and TBME, and hydrolysis in phosphate buffer (50 mM, pH 7) and in the presence of small amounts of added water in toluene, TBME, or dichloromethane all displayed negligible enantioselectivities ($E < 10$), as measured by the enantiomer ratio. The highest enantioselectivity ($E = 36$) was observed for the aminolysis of *rac*-**2** with *n*-hexylamine and lipase PS-C II (*Burkholderia cepacia* lipase on

Toyonite 200M) in DIPE (Scheme 2). Unfortunately, the aminolysis proceeded too slowly (20% conversion after 1 day in DIPE with 100 mg mL^{-1} of lipase PS-C II) to be of any practical use and the kinetic resolution of *rac*-**2** was not further studied.
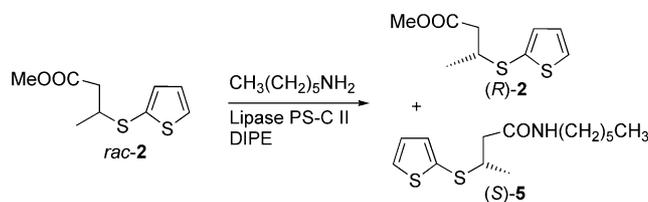
Scheme 2. Kinetic resolution of *rac*-**2** by aminolysis; absolute configurations unconfirmed.

The enantioselective acylation reactions of 6-methyl-5,6-dihydro-4*H*-thieno[2,3-*b*]thiopyran-4-ol (*rac-cis*-**3**) and the corresponding 7,7-dioxide (*rac-cis*-**4**) with lipases were studied next. The lipase-catalyzed enantioselective acylation reactions of some 3-substituted cyclohexanols have previously been reported.[14] In these reactions, the 1*R*,3*S* enantiomer (one of the *cis* isomers), in which the substituents in the major conformation are equatorial, was favorably *O*-acylated in the presence of lipase PS and CAL-B.[14a,14b] The corresponding *trans* isomers reacted slowly or did not react at all as the substituent at C-3 prefers to be equatorial forcing the HO group to be axial. Molecular modeling studies of CAL-B with the more thoroughly studied 2-substituted cyclohexanols give more support to the importance of conformation on reactivity.[15] On the basis of the above, the absolute configuration 4*R*,6*S* was elucidated for the ester products **6**–**8**. With the enantiomers of *rac-cis*-**4** the assigned configuration was in accord with those disclosed by a patent that gives the absolute configurations for the peaks in HPLC chromatograms, thus supporting our elucidation.[16] Much work was performed on *rac-cis*-**3** as the substrate as it appears earlier in the synthetic protocol of dorzolamide and is more soluble than *rac-cis*-**4** in organic solvents. Thus, *rac-cis*-**3** (*de* 91%, 0.05 M) was first subjected to lipase screening for the acylation reaction with vinyl butanoate (0.1 M) in TBME in the presence of each lipase preparation (50 mg mL^{-1}, route A, Scheme 3). All the lipases gave the same enantiopreference. *Burkholderia cepacia* lipase in general (entries 1–3) and lipase PS-D (lipase PS preparation on Celite; entry 3) in particular displayed high enantioselectivity and gave the best conversion, that is, reactivity, after a certain time (Table 1). Reducing the amount of lipase PS-D to 25 mg mL^{-1} did not affect the reactivity (entries 3 and 4). When the acylation of *rac-cis*-**3** with vinyl butanoate and lipase PS-D was screened in various solvents, the enantioselectivities varied greatly as shown by the *E* values of >200 in TBME, 111 in DIPE, 104 in toluene, 82 in cyclohexane, 40 in acetonitrile, and 25 in acetone. TBME, which best dissolves *rac-cis*-**3** and is also free from peroxides, was chosen for further optimization.
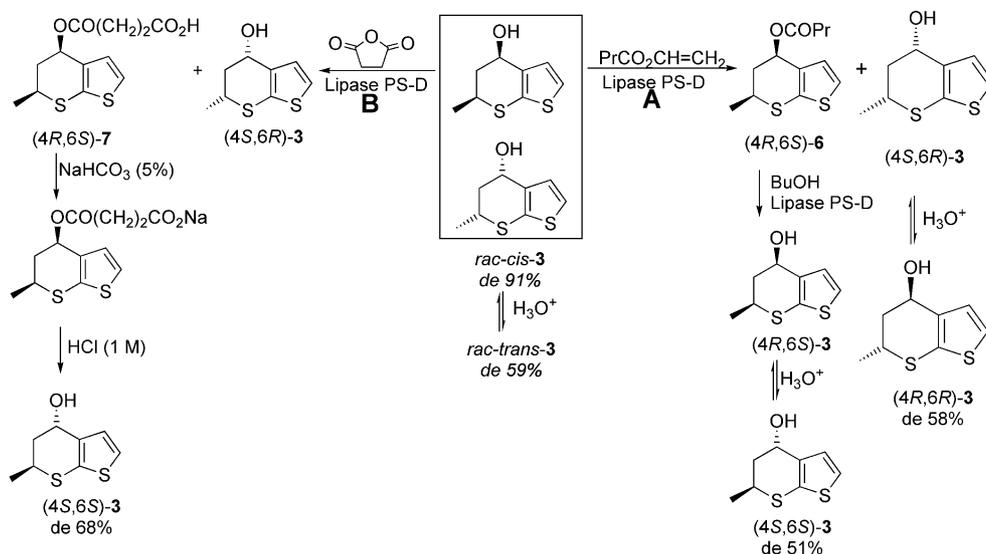
Scheme 3. Kinetic resolution of *rac-cis*-**3** by acylation and epimerization.

Table 1. Lipase screening (50 mg mL^{-1}) for the acylation of *rac-cis*-**3** (0.05 M) with vinyl butanoate (0.1 M) in TBME at room temperature.

Entry	Enzyme	Time [h]	% Conv.	% ee for (4R,6S)-**6**	E
1	lipase PS powder	6	41	97	160 ± 38
2	lipase PS-C II	0.5	45	95	83 ± 11
3	lipase PS-D	0.5	51	97	>200
4	lipase PS-D[a]	0.5	51	97	>200
5	lipase PS-D[b]	0.5	26	99	>200
6	CAl-B	6	13	83	12 ± 0.16
7	CRl	6	52	86	54 ± 16
8	lipase Tl IM	6	47	78	16 ± 0.2
9	lipase AK-C	6	52	92	137 ± 18

[a] 25 mg mL^{-1} of lipase PS-D. [b] 10 mg mL^{-1} of lipase PS-D.

In the lipase-catalyzed acylation reaction, the structure of the acyl donor is often critical to the enantioselectivity and reactivity. When *rac-cis*-**3** was subjected to acylation with various vinyl esters and acid anhydrides excellent enantioselectivities and reactivities were observed with lipase PS-D in TBME except with glutaric anhydride (entry 7, Table 2). Changing the acyl group from acetate to laurate and the use of dicarboxylic acid anhydrides allowed us systematically to tune the hydrophobicity of the 4*R*,6*S* ester obtained relative to the unreacted alcohol enantiomer (4*S*,6*R*)-**3** (routes A and B, Scheme 3). As Gutmann et al.[17] first showed, half esters of succinic acid are often water-soluble, the property that allows the separation of the resolved products by extraction after kinetic resolution is completed. Accordingly, succinic anhydride was expected to be the most interesting acyl donor for the kinetic resolution of *rac-cis*-**3** (route B). As a drawback, elimination of water from the alcohol counterpart was significant during the kinetic resolution whereas it was insignificant with vinyl butanoate as the acyl donor. More seriously, aqueous extraction led to the isolation of (4*S*,6*R*)-**3** containing around 8%

of succinate **7**, and aqueous work-up of the half ester finally gave an epimerized mixture of (4*S*,6*S*)- and (4*R*,6*S*)-**3** with a *de* 68%. For these reasons we decided to continue optimization with vinyl butanoate rather than with succinic anhydride. Chemical acylation of *rac-cis*-**3** was not detected with any of the acyl donors studied.

Table 2. Screening of acyl donors (0.1 M) for the acylation of *rac-cis*-**3** (0.05 M) in the presence of lipase PS-D (25 mg mL^{-1}) in TBME at room temperature.

Entry	Acyl donor	Time [h]	% Conv.	% ee for (4R,6S)-**P**[a]	E
1	vinyl acetate	0.5	51	94	>200
2	vinyl butanoate	0.5	43	99	>200
3	vinyl laureate	1	48	99	>200
4	butanoic anhydride	0.5	45	98	>200
5	hexanoic anhydride	1	41	98	>200
6	succinic anhydride	0.5	46	94	134 ± 2
7	glutaric anhydride	1	10	97	80 ± 6

[a] Acetate, hexanoate and laurate esters (the corresponding product **P**) were produced in place of butanoate **6** and the monoglutarate ester in place of monosuccinate **7** in Scheme 3.

rac-cis-**3** dissolves in TBME up to 0.8 M in solution. Next concentration effects were studied by subjecting *rac-cis*-**3** (0.05–0.8 M) to acylation with vinyl butanoate (1–2 equiv.) in the presence of lipase PS-D (25 mg mL^{-1}) in TBME (Table 3). The reactivity dropped considerably with increasing substrate concentration (entries 3, 5, and 6) although 50% conversion was finally reached in every case. Rather than trying to increase the reactivity by increasing the amount of enzyme we chose to keep the enzyme content low and used 0.1 M of the substrate in preparative-scale kinetic resolution. We also found that with the 0.1 M racemate the amount of vinyl butanoate had practically no effect on either the reactivity or the enantioselectivity (entries 2–4). Finally, 5.00 g of *rac-cis*-**3** (0.1 M) with 1.2 equiv. of vinyl

butanoate were effectively transformed into (4R,6S)-**6** and (4S,6R)-**3** in enantio- and diastereopure forms as described in the Exp. Section. To prepare (4R,6S)-**3** the enzymatically produced (4R,6S)-**6** has to be deacylated under mild conditions in which epimerization and thus the formation of an equilibrium mixture of 4R,6S and 4S,6S isomers can be prevented. For this purpose, the alcoholysis of (4R,6S)-**6** with simple alcohols and lipase PS-D was studied (entries 1–4, Table 4). We chose alcoholysis with *n*-butanol for further studies (route A, Scheme 3). As alcohols are known to cause lipase inhibition,[18] low alcohol contents (0.1 M substrate and 0.2 or 0.3 M alcohol) gave the best conversions with time (compare entries 4 and 6 with entry 7). Alcoholysis in hexane (entry 8) and in toluene (entry 9) gave low reactivity. The use of an elevated temperature (47 °C) practically allowed completion of the reaction in 24 h (entry 5).

Table 3. Concentration effects for the acylation of *rac-cis*-**3** with vinyl butanoate (1.2 equiv.) in the presence of lipase PS-D (25 mg mL^{-1}) in TBME at room temperature.

Entry	[Substrate] [M]	Time [h]	% Conv.	% ee for (4R,6S)-**6**	% ee for (4S,6R)-**3**	E
1	0.05[a]	0.5	51	99	97	>200
2	0.1[a]	1	49	98	93	>200
3	0.1	1	49	97	93	>200
4	0.1[b]	1	47	99	88	>200
5	0.5	4	49	99	96	>200
6	0.8	5	50	96	97	>200

[a] With 2 equiv. of vinyl butanoate. [b] With 1 equiv. of vinyl butanoate.

Table 4. Alcoholysis of (4R,6S)-**6** (ee 95%, 0.1 M) with alcohols in organic solvent in the presence of lipase PS-D (50 mg mL^{-1}) over 24 h.

| Entry	Alcohol	Substrate/ROH [mol:mol]	Solvent	% Conv.	% ee for (4R,6S)-**3**
1	MeOH	1:2	TBME	88	99
2	EtOH	1:2	TBME	84	99
3	*i*PrOH	1:2	TBME	86	99
4	BuOH	1:2	TBME	90	99
5	BuOH[a]	1:2	TBME	97	96
6	BuOH	1:3	TBME	89	99
7	BuOH	1:6	TBME	70	99
8	BuOH	1:2	hexane	37	99
9	BuOH	1:2	toluene	10	99

[a] Reaction temperature 47 °C.

As reported[3] and discussed above, the 4-position in *cis*-**3** is available for epimerization, and an equilibrium is established between the *cis* and *trans* isomers in slight favor of the more stable *trans* isomer (Scheme 3). When *rac-trans*-**3** (de 59%) was prepared from *rac-cis*-**3** under acidic conditions and the epimerized mixture (0.1 M) was subjected to acylation with vinyl butanoate (0.2 M) and lipase PS-D (25 mg mL^{-1}) in TBME, only 6% of the *trans* isomer was converted after 1 h in accord with the importance of the conformation on reactivity.[14] The reaction was not enantioselective at all, as shown by an E value of 1.4, which

indicates that kinetic resolution cannot be used for the preparation of *trans* enantiomers. Accordingly, we next turned our attention to the epimerization of (4S,6R)- and (4R,6S)-**3** to prepare (4R,6R)- and (4S,6S)-**3**, respectively (see Schemes 4 and 5). Epimerization was performed in a 1:1 biphasic system of TBME/H$_2$SO$_4$ (1 M). With (4R,6S)-**3** (ee 99%) as the starting material, the unepimerized counterpart in the mixture (de 51%) was transformed into (4R,6S)-**6** with lipase PS-D and vinyl butanoate in TBME, allowing the purification of the unreacted (4S,6R)-**3** (ee 99%, de 96%; Scheme 4). The preparation of (4R,6R)-**3** turned to be more complicated. The epimerized mixture [(4R,6R)- and (4S,6R)-**3**, de 58%] contains the less reactive *cis* enantiomer but the *trans* enantiomer, although having the more reactive 4R center, was acylated slowly. Nevertheless, lipase PS-D-catalyzed acylation with vinyl butanoate in TBME was used to produce first a mixture of butanoates, and then highly diastereoselective lipase PS-D-catalyzed alcoholysis of (4R,6R)-**6** with *n*-butanol yielded (4R,6R)-**3** (Scheme 5). Although *rac-cis*-**4**, even as a 0.05 M solution, is poorly soluble in TBME, the lipase PS-D-catalyzed acylation of *rac-cis*-**4** (0.05 M) with vinyl acetate (1.2 equiv.) proceeded with good enantioselectivity, as can be seen from the high enantiopurity of the product ester at 51% conversion (entry 1, Table 5; Scheme 6). Vinyl acetate was used in place of vinyl butanoate simply for analytical reasons (the enantiomers of the butanoate ester were not base-line separated by the GC method used). Toluene and especially toluene/co-solvent (9:1) mixtures (toluene/DMSO as an exception, entry 8) dissolved *rac-cis*-**4** better than TBME, which allowed the acylation reactions with vinyl acetate to proceed with excellent enantioselectivity (entries 2–7). Owing to the volatility of acetone, toluene/acetone (9:1) was chosen as the solvent for the preparative-scale kinetic resolution of *rac-cis*-**4**. The solvent system was able to dissolve the substrate up to a 0.3 M solution without much effect on reactivity and with no effect on enantioselectivity (entries 5–7). Finally, (4R,6S)- and (4S,6R)-**4** were effectively prepared by lipase PS-D-catalyzed acylation of *rac-cis*-**4** (0.1 M) with vinyl acetate (1.2 equiv.) followed by the alcoholysis of (4R,6S)-**8** with *n*-butanol, as described in the Exp. Section.
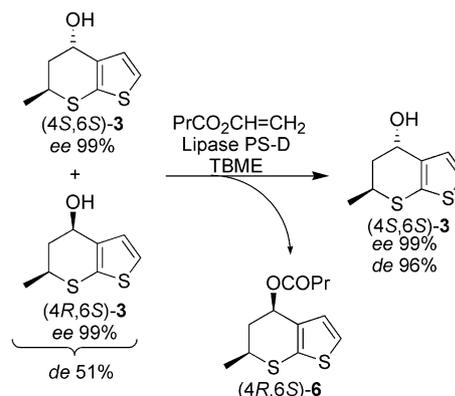
Scheme 4. Preparation of (4S,6R)-**3**.
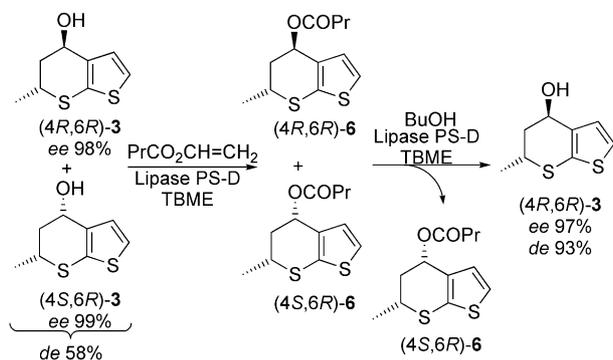
Scheme 5. Preparation of (4*R*,6*R*)-**3**.

Table 5. Solvent effect on the acylation of *rac-cis*-**4** (0.05 M) with vinyl acetate (2 equiv.) in the presence of lipase PS-D (10 mg mL^{-1}) at room temperature.

Entry	Solvent	Time [h]	% *ee* for (4*R*,6*S*)-**8**	% Conv.	*E*
1	TBME[a]	1	95	51	–
2	toluene[a]	2	96	51	–
3	toluene/*tert*-amyl alcohol (9:1)	2	96	51	>200
4	toluene/2-methyltetra-hydrofuran (9:1)	2	95	51	>200
5	toluene/acetone (9:1)	3	96	50	>200
6	toluene/acetone (9:1)[b]	4	96	50	>200
7	toluene/acetone (9:1)[c]	4	97	50	>200
8	toluene/DMSO (9:1)	2	96	6	49 ± 16

[a] The substrate was not fully soluble; the *E* value is not reliable. [b] Substrate concentration: 0.2 M. [c] Substrate concentration: 0.3 M.
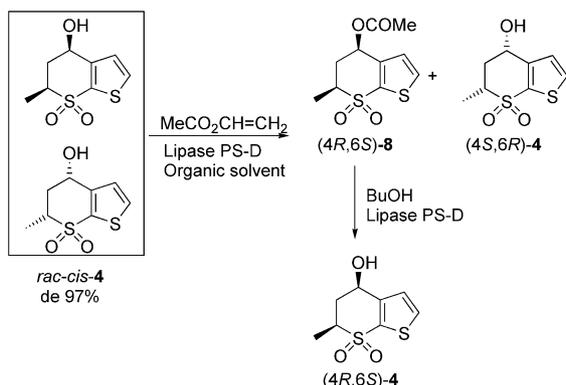
Scheme 6. Kinetic resolution of *rac-cis*-**4** by acylation.

Conclusions

The kinetic resolution of dorzolamide intermediates *rac*-**2**, *rac-cis*-**3**, and *rac-cis*-**4** have been studied in the presence of lipases mainly in organic solvents. We have shown that lipase PS-D-catalyzed asymmetric acylation reactions of *rac-cis*-**3** and *rac-cis*-**4** with vinyl esters are effective and highly enantioselective reactions that allow the preparation of the ester enantiomers with the desired 4*R*,6*S* configuration. Lipase PS-D-catalyzed alcoholysis with *n*-butanol

proved to be a mild method for transforming the ester enantiomers into the corresponding (4*R*,6*S*)-**3** and (4*R*,6*S*)-**4**. Thus, with the published syntheses of dorzolamide in mind, we have prepared the intermediates, which, by epimerization followed by the Ritter reaction or by conventional inversion of the configuration, can be used to introduce the amino group at C-4 with the *S* configuration of the dorzolamide (Scheme 1). Epimerization of the *cis* enantiomers produced also allowed us to prepare the *trans* enantiomers of **3**, although the procedure needed additional enzymatic purifications steps. Although not studied here, the epimerization method evidently also allows the preparation of the *trans* stereoisomers of **4** from the corresponding *cis* enantiomers. Our work shows that the kinetic resolution of *rac*-**2** was less promising.

Experimental Section

General: All solvents were of the highest analytical grade and were dried with molecular sieves prior to use. The racemic starting materials *rac*-**2**, *rac-cis*-**3**, and *rac-cis*-**4** were generous gifts from PCAS Finland Oy. The products of the enzymatic reactions in racemic forms were prepared by standard methods for analytical purposes. Lipase B from *Candida antarctica* (CAL-B, Novozym 435) and lipase TL IM from *Thermomyces lanuginosus* were purchased from Novozymes and *Candida rugosa* lipase (CRL) from Sigma. Lipase AK from *Pseudomonas fluorescens* and *Burkholderia cepacia* lipase preparations (lipase PS powder, lipase PS-C II on Toyonite 200M and lipase PS-D on Celite) were from Amano Pharmaceuticals.

HRMS were recorded with a ZabSpec-oaTof instrument. The solution-state ^{1}H and ^{13}C NMR spectra were recorded with a Bruker Avance 500 spectrometer equipped with a BBO-5 mm-Zgrad probe operating at 500.13 and 125.77 MHz, respectively. Tetramethylsilane (δ = 0.00 ppm) was used as the reference for both the ^{1}H and ^{13}C NMR spectra. All NMR spectra were recorded at +25 °C. The correct assignments of the chemical shifts were confirmed when necessary by two-dimensional correlation measurements attained by ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC, NOESY, or 1D NOESY experiments. Optical rotations were measured by using a Perkin–Elmer 341 Polarimeter against the sodium D line, the values for $[a]_D^{25}$ being in units of 10^{-1} deg cm^{-2} g^{-1}. Melting points were measured with a Gallenkamp apparatus and are uncorrected. Analytical thin-layer chromatography (TLC) was carried out with Merck Kieselgel 60F$_{254}$ sheets. Preparative chromatographic separations were performed by column chromatography on Merck Kieselgel 60 (0.063–0.200 μm). All reactions were performed at room temperature (23–24 °C) unless otherwise stated.

Small-Scale Enzymatic Reactions: In a typical procedure, *rac*-**2** (0.05 M), *rac-cis*-**3** (0.05–0.8 M), or *rac-cis*-**4** (0.05–0.3 M) was dissolved in solvent (2 mL) and one of the lipase preparations was added. The addition of a nucleophile (2 equiv.) or an acyl donor (1–2 equiv.) started the reaction The alcoholysis of (4*R*,6*S*)-**6** (0.1 M) and (4*R*,6*S*)-**8** (0.1 M) was performed with an alcohol (0.2–0.6 M) in TBME in the presence of lipase PS-D. The reactions proceeded while shaking at 170 rpm. The progress of the reactions was followed by removing samples (100 μL) at intervals, filtering off the enzyme, and analyzing the samples by GC or HPLC. When HPLC analysis was used the samples withdrawn from the reaction mixture were further diluted with the eluent used in the HPLC analysis. The reactions of *rac*-**2** were analyzed by HPLC with a Daicel Chi-

ralcel OD column (250 × 46 mm ID), those of *rac-cis*-**3** by GC with a Supelco Beta Dex™ 120 capillary column (30 m × 0.25 mm × 0.25 µm), and those of *rac-cis*-**4** by GC with a Varian capillary column (25 m × 0.25 mm) of WCOT fused silica with CP-Chirasil-DEX CB as the stationary phase. When succinic or glutaric anhydride was used as the acyl donor the half acid in the sample (200 µL) was derivatized before analysis with a solution (20 µL) of 2 M (trimethylsilyl)diazomethane in hexane in the presence of methanol (20 µL) and analysis was performed by HPLC equipped with a Daicel Chiracel OD-H column (250 × 46 mm ID). The HPLC method was also used to monitor the epimerization reactions of *rac-cis*-**3** because it affords base-line separation of all four diastereomers of compound **3**. An HPLC analytical method was also developed for *rac-cis*-**4** to confirm the absolute configuration of the enantiomers by comparing the order of peaks and the configuration assigned in an existing patent.[16] The determination of *E* was based on the equation given below by using linear regression {*E* as the slope of the line ln[(1 – *c*)(1 – *ee*$_S$)] vs. ln[(1 – *c*)(1 + *ee*$_S$)]}.[19]

$$E = \ln[(1 - c)(1 - ee_S)]/\ln[(1 - c)(1 + ee_S)] \text{ with } c = ee_S/(ee_S + ee_P)$$

Preparation of (4S,6R)- and (4R,6S)-6-Methyl-5,6-dihydro-4H-thieno[2,3-*b*]thiopyran-4-ols: Compound *rac-cis*-**3** (5.00 g, 26.84 mmol, m.p. 96–97 °C, *de* 91%) was dissolved in TBME (264 mL) and lipase PS-D (6.50 g) and vinyl butanoate (3.67 g, 4.08 mL, 32.21 mmol) were added. The reaction was stopped at 48% conversion after 1 h by filtering off the enzyme and evaporating the solvent. Purification by column chromatography [silica, ethyl acetate/hexane (3:7)] yielded first the ester (4R,6S)-**6** as a light-yellow oil (3.02 g, 11.77 mmol, yield 91%, *ee* 95%, *de* 78%). [*a*]$_D^{25}$ = –80.0 (*c* = 1.0, CHCl$_3$). ^{1}H NMR (CDCl$_3$, 500 MHz): δ = 0.97 (t, *J* = 7.5 Hz, 3 H, COCH$_2$CH$_2$C*H*$_3$), 1.43 (d, *J* = 6.5 Hz, 3 H, 6-CH$_3$), 1.69 (sext, *J* = 7.5 Hz, 2 H, OCOCH$_2$C*H*$_2$CH$_3$), 1.97–2.04 (m, 1 H, 5-H), 2.34 (t, *J* = 7.5 Hz, 2 H, OCOC*H*$_2$CH$_2$CH$_3$), 2.51 (ddd, *J* = 13.5, *J* = 6.0, *J* = 2.0 Hz, 1 H, 5-H), 3.57–3.61 (m, 1 H, 6-H), 6.03 (dd, *J* = 8.5, *J* = 6.0 Hz, 1 H, 4-H), 6.86 (d, *J* = 5.5 Hz, 1 H, 3-H), 7.04 (d, *J* = 5.5 Hz, 1 H, 2-H) ppm. ^{13}C NMR (CDCl$_3$, 126 MHz): δ = 13.69 (OCOCH$_2$CH$_2$*C*H$_3$), 18.54 (OC-OCH$_2$*C*H$_2$CH$_3$), 20.81 (6-*C*H$_3$), 36.50 (OCO*C*H$_2$CH$_2$CH$_3$), 37.20 (C-6), 37.56 (C-5), 67.76 (C-4), 121.55 (C-3), 126.86 (C-2), 130.65 (C-8), 132.44 (C-9), 173.42 (O*C*OCH$_2$CH$_2$CH$_3$) ppm. HRMS: calcd. for C$_{12}$H$_{16}$S$_2$O$_2$ [M]$^+$ 256.05917; found 256.05950. MS: *m/z* (%) = 256 (91), 186 (7), 169 (100), 153 (87), 143 (8), 136 (13), 71 (27).

For deprotection, (4R,6S)-**6** (1.00 g, 3.91 mmol, *ee* 95%) was dissolved in TBME (39 mL) and *n*-butanol (0.58 g, 0.71 mL, 7.80 mmol) and lipase PS-D (1.95 g) were added. The reaction was shaken at 47 °C for 30.5 h until 92% conversion was reached. The enzyme was filtered off and the solvent evaporated. Purification as above yielded (4R,6S)-**3** as a white solid (0.61 g, 3.30 mmol, 84%, *ee* 99%, *de* 99%). [*a*]$_D^{25}$ = –214.4 (*c* = 1.0, CHCl$_3$); m.p. 98–99 °C. ^{1}H NMR (CDCl$_3$, 500 MHz): δ = 1.41 (d, *J* = 7.0 Hz, 3 H, 6-C*H$_3$*), 1.89 (ddd, *J* = 21.0, *J* = 10.0, *J* = 2.0 Hz, 1 H, 5-H), 2.44 (ddd, *J* = 13.0, *J* = 5.5, *J* = 2.0 Hz, 1 H, 5-H), 3.53–3.60 (m, 1 H, 6-H), 4.86 (dd, *J* = 10.0, *J* = 6.0 Hz, 1 H, 4-H), 7.04 (d, *J* = 5.0 Hz, 1 H, 3-H), 7.08 (d, *J* = 5.0 Hz, 1 H, 2-H) ppm. ^{13}C NMR (CDCl$_3$, 126 MHz): δ = 20.84 (6-CH$_3$), 37.68 (C-6), 42.43 (C-5), 67.09 (C-4), 121.55 (C-3), 126.63 (C-2), 131.08 (C-8), 135.16 (C-9). HRMS: calcd. for C$_8$H$_{10}$S$_2$O [M]$^+$ 186.01731; found 186.01720. MS: *m/z* (%) = 186 (88), 169 (7), 153 (28), 144 (100), 116 (9), 110 (28), 84 (7).

The unreacted alcohol (4S,6R)-**3** from the resolution mixture was eluted after (4R,6S)-**6** as a white-grey solid (2.33 g, 12.50 mmol,

89%, *ee* 98%, *de* 98%). [*a*]$_D^{25}$ = +213.4 (*c* = 1.0, CHCl$_3$); m.p. 99–100 °C.

Preparation of (4R,6R)- and (4S,6S)-6-Methyl-5,6-dihydro-4H-thieno[2,3-*b*]thiopyran-4-ols: Compound (4S,6R)-**3** (*ee* 98%, *de* 98%) or (4R,6S)-**3** (*ee* 99%, *de* 99%) (0.50 g, 2.69 mmol) was dissolved in TBME (2.70 mL) and 1 M H$_2$SO$_4$ (2.70 mL) was added. The biphasic system was vigorously shaken at room temperature for 24 h for epimerization before the organic phase was separated and washed with brine and dried with Na$_2$SO$_4$. Evaporation yielded (4R,6R)-**3** (*ee* 98%, *de* 58%) or (4S,6S)-**3** (*ee* 99%, *de* 51%), respectively. The progress of the epimerization was followed by HPLC analysis (Chiralcel OD-H column, 1% isopropyl alcohol in hexane, 0.7 mL min^{-1} flow, 238 nm).

Compound (4S,6S)-**3** (*ee* 99%, *de* 51%) (0.07 g, 0.41 mmol) was dissolved in TBME (4 mL) and lipase PS-D (0.10 g) and vinyl butanoate (0.09 g, 0.10 mL, 0.81 mmol) were added for diastereomeric purification. After 1 h, purification as before yielded (4S,6S)-**3** as a white-pink solid (0.04 g, 0.23 mmol, 57%, *ee* 99%, *de* 96%). [*a*]$_D^{25}$ = –222.5 (*c* = 1.1, CHCl$_3$); m.p. 81–82 °C). ^{1}H NMR (CDCl$_3$, 500 MHz): δ = 1.42 (d, *J* = 6.5 Hz, 3 H, 6-CH$_3$), 1.80–1.86 (m, 1 H, 5-H), 2.29 (dt, *J* = 14.5, *J* = 2.5 Hz, 1 H, 5-H), 3.53–3.60 (m, 1 H, 6-H), 4.85 (t, *J* = 3.0 Hz, 1 H, 4-H), 6.99 (d, *J* = 5.5 Hz, 1 H, 3-H), 7.05 (d, *J* = 5.5 Hz, 1 H, 2-H) ppm. ^{13}C NMR (CDCl$_3$, 126 MHz): δ = 20.33 (6-CH$_3$), 33.20 (C-6), 40.32 (C-5), 63.64 (C-4), 121.64 (C-3), 127.88 (C-2), 133.14 (C-8), 133.47 (C-9) ppm. HRMS: calcd. for C$_8$H$_{10}$S$_2$O [M]$^+$ 186.01731; found 186.01720. MS: *m/z* (%) = 186 (88), 169 (7), 153 (28), 144 (100), 116 (9), 110 (28), 84 (7).

Compound (4R,6R)-**3** (*ee* 99%, *de* 58%; 0.25 g, 1.34 mmol) was dissolved in TBME (13 mL) and lipase PS-D (0.33 g) and vinyl butanoate (0.30 g, 0.34 mL, 2.68 mmol) were added. The normal work-up after 21 h yielded first (4R,6R)-**6** as a light-yellow liquid (0.25 g, 0.98 mmol, *ee* 96%, *de* 70%). For deprotection, (4R,6R)-**6** (0.15 g, 0.58 mmol) was dissolved in TBME (6 mL) and *n*-butanol (0.08 g, 0.11 mL, 1.17 mmol) and lipase PS-D (0.44 g) were added. The reaction was shaken at 47 °C for 3 d. The reaction was stopped by filtering off the enzyme and then the solvent was evaporated. Purification as before yielded (4R,6R)-**3** as a white solid (0.05 g, 0.28 mmol, 48%, *ee* 97%, *de* 93%). [*a*]$_D^{25}$ = +229.7 (*c* = 1.0, CHCl$_3$); m.p. 80–81 °C.

Preparation of (4S,6R)- and (4R,6S)-4-Hydroxy-6-methyl-5,6-di-hydro-4H-thieno[2,3-*b*]thiopyran 7,7-Dioxide: *rac-cis*-**4** (2.00 g, 9.16 mmol, m.p. 123–125 °C, *de* 97%) was dissolved in the mixture toluene/acetone (9:1; 90 mL) and lipase PS-D (0.91 g) and vinyl acetate (1.58 g, 1.69 mL, 18.32 mmol) were added. The reaction was stopped at 50% conversion after 3 h by filtering off the enzyme and then evaporating the solvent. Purification by column chromatography [silica, ethyl acetate/hexane (1:1)] yielded first the ester (4R,6S)-(**8**) as a white solid (0.97 g, 3.72 mmol, 87%, *ee* 97%, *de* 99%). [*a*]$_D^{25}$ = –18.5 (*c* = 1.0, CHCl$_3$); m.p. 111–113 °C. ^{1}H NMR (CDCl$_3$, 500 MHz): δ = 1.53 (d, *J* = 7.0 Hz, 3 H, 6-C*H$_3$*), 2.15 (s, 3 H, OCOC*H$_3$*), 2.64 (dddd, *J* = 14.5, *J* = 5.5, *J* = 2.5 Hz, 1 H, 5-H), 2.62–2.63 (m, 1 H, 5-H), 3.48–3.55 (m, *J* = 2.5 Hz, 1 H, 6-H), 6.03 (dd, *J* = 9.0, *J* = 6.0 Hz, 1 H, 4-H), 6.95 (d, *J* = 5.0 Hz, 1 H, 3-H), 7.58 (d, *J* = 5.0 Hz, 1 H, 2-H) ppm. ^{13}C NMR (CDCl$_3$, 126 MHz): δ = 11.47 (6-C*H$_3$*), 21.02 (OCOC*H$_3$*), 35.18 (C-5), 55.84 (C-6), 66.45 (C-4), 126.32 (C-3), 130.89 (C-2), 136.75 (C-8), 141.73 (C-9), 170.27 (O*C*OCH$_3$) ppm. HRMS: calcd. for C$_8$H$_{10}$S$_2$O$_4$ [M]$^+$ 260.01770; found 260.01740. MS: *m/z* (%) = 260 (18), 217 (47), 174 (28), 154 (32), 135 (26), 111 (22), 91 (12), 69 (10).

For deprotection, (4R,6S)-**8** (0.5 g, 1.92 mmol, *ee* 97%) was dissolved in the mixture toluene/acetone (9:1; 19 mL) and *n*-butanol

(0.28 g, 0.35 mL, 3.84 mmol) and lipase PS-D (0.48 g) were added. The reaction was shaken at 47 °C. The reaction was stopped after 23 h at 95% conversion by filtering off the enzyme and then the solvent was evaporated. Purification by column chromatography [silica, ethyl acetate/hexane (1:1)] gave (4R,6S)-**4** as a white solid (0.35 g, 1.60 mmol, 83%, *ee* 99%, *de* 99%). $[a]_D^{25}$ = –75.4 (*c* = 1.0, CHCl$_3$); m.p. 154–155 °C. ^{1}H NMR (CDCl$_3$, 500 MHz): δ = 1.50 (d, J = 6.5 Hz, 3 H, 6-CH_3), 2.36 (ddd, J = 14.5, J = 12.0, J = 10.5 Hz, 1 H, 5-H), 2.47 (ddd, J = 14.0, J = 5.5, J = 2.5 Hz, 1 H, 5-H), 2.55 (br. s, 1 H, OH), 3.40–3.47 (m, 1 H, 6-H), 4.87 (dd, J = 9.0, J = 5.0 Hz, 1 H, 4-H), 7.16 (d, J = 5.0 Hz, 1 H, 3-H), 7.58 (d, J = 5.0 Hz, 1 H, 2-H) ppm. ^{13}C NMR (CDCl$_3$, 126 MHz): δ = 11.15 (6-CH$_3$), 39.35 (C-5), 56.37 (C-6), 66.18 (C-4), 126.57 (C-3), 130.90 (C-2), 135.02 (C-8), 146.46 (C-9) ppm. HRMS: calcd. for $C_8H_{10}S_2O_3$ [M]$^+$ 218.00714; found 218.00660. MS: *m/z* (%) = 218 (38), 176 (14), 158 (30), 139 (12), 128 (16), 111 (100), 83 (11), 69 (9).

The unreacted alcohol (4S,6R)-**4** from the resolution mixture was eluted after (4R,6S)-**8** as a white solid (0.89 g, 4.08 mmol, 89%, yield 89%, *ee* 96%, *de* 99%). $[a]_D^{25}$ = +69.6 (*c* = 1.0, CHCl$_3$); m.p. 151–152 °C.

Supporting Information (see also the footnote on the first page of this article): HPLC chromatograms for the diastereomers of compound **3**.

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