catalysts

MDPI

Article

Chemo-Enzymatic Synthesis of Enantiopure β-Antagonist (*S*)-Betaxolol

Susanne Hansen Troøyen and Elisabeth Egholm Jacobsen *

Department of Chemistry, Norwegian University of Science and Technology, Høgskoleringen 5, 7491 Trondheim, Norway
* Correspondence: elisabeth.e.jacobsen@ntnu.no

Abstract: The β-blocker (*S*)-betaxolol has been synthesized in 99% enantiomeric excess (*ee*) from the commercially available precursor 4-(2-hydroxyethyl)phenol. The racemic chlorohydrin 1-chloro-3-(4-(2-(cyclopropylmethoxy)ethyl)phenoxy)propan-2-ol was esterified with vinyl acetate catalyzed by lipase B from *Candida antarctica*, which gave the *R*-chlorhydrin (*R*)-1-chloro-3-(4-(2-(cyclopropylmethoxy) ethyl)phenoxy)propan-2-ol in 99% *ee* with 38% yield. The enantiomeric excess of the *R*-chlorohydrin was retained in an amination reaction with isopropylamine in methanol to yield (*S*)-betaxolol in 99% *ee* and with 9% overall yield. We are under way to improve the yield.

Keywords: (*S*)-betaxolol; enantiopure building blocks; *Candida antarctica* lipase B; chiral chromatography

check for updates

Citation: Troøyen, S.H.; Jacobsen, E.E. Chemo-Enzymatic Synthesis of Enantiopure β-Antagonist (*S*)-Betaxolol. *Catalysts* **2022**, *12*, 1645. https://doi.org/10.3390/catal12121645

Academic Editors: Pawel Borowiecki and Dominik Koszelewski

Received: 9 November 2022
Accepted: 9 December 2022
Published: 15 December 2022

1. Introduction

Betaxolol (brand names Kerlone and Optipres-S) is a selective β1-receptor antagonist which may be used to treat hypertension, though it is more commonly used topically as an anti-glaucoma agent [1]. Glaucoma is a dysfunction or damage affecting the optic nerve, leading to gradual loss of eyesight, which is often caused by high intraocular pressure [2]. Glaucoma is the leading cause of irreversible blindness in the world, and it has been estimated that over 70 million people live with glaucoma worldwide [3]. The most active enantiomer (the eutomer) of betaxolol is the (*S*)-enantiomer, see Figure 1 [4].

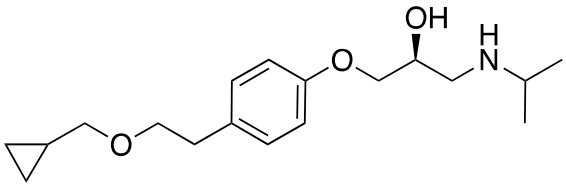


Figure 1. (*S*)-Betaxolol ((*S*)-1-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-3-(propan-2-ylamino)propan-2-ol.

In 1992, the US Food and Drug Administration (FDA) announced a policy demanding drug manufacturers to test the pharmacokinetic activity of both enantiomers of a drug separately in order to avoid negative side effects caused by one enantiomer [5]. This has led to a high level of interest towards the development of economical and sustainable methods for the production and analysis of enantiopure drugs, and in many cases a "chiral switch" from racemic to enantiopure drugs [6].

An early study reporting the synthesis of enantiopure (*S*)-betaxolol in addition to a screening of its β-receptor blocking activity was performed by Manoury et al. in 1987 [7]. A screening of several para-substituted phenoxypropanolamines was performed, and it was discovered that (*S*)-betaxolol had great potential as a β-blocker, with potency exceeding the established drugs metoprolol and propranolol. (*S*)-Betaxolol was synthesized from 4-(2-(cyclopropylmethoxy)ethyl)phenol using (*S*)-2-phenyl-3-isopropyl-5-(hydroxylmethyl)oxazolidinyl tosylate, which is a chiral reagent that already possesses

the desired stereoconfiguration. This compound is not commercially available. Joshi et al. developed a pathway to obtain enantiopure (*S*)-betaxolol in 2005, which involves a hydrolytic kinetic resolution of 2-((4-(2-(cyclopropylmethoxy)ethyl)phenoxy)methyl)oxirane using Jacobsen's catalyst (*R*,*R*-salen Co(III)), an asymmetric organometallic catalyst [8]. The epoxide was synthesized from 4-(2-hydroxyethyl)phenol in six steps, including phenol protection with a benzyl group, a Simmons–Smith cyclopropanation and deprotection by hydrogenation over Raney Nickel. The overall yield from the starting material to (*S*)-betaxolol in 99% *ee* was 17%. Wang et al. reported a direct alkylation of the primary alcohol of 4-(2-hydroxyethyl)phenol without protection of the phenol [9]. A different approach to (*S*)-betaxolol was published by Datta et al. in 2006 [10]. A Pd-catalyzed Heck arylation of ((vinyloxy)methyl)cyclopropane with 1-chloro-4-nitrobenzene was performed. After some modifications, 4-(2-(cyclopropylmethoxy)ethyl)phenol was formed, and the addition of the chiral epoxide (*R*)-3-isopro-pylamino-1,2-epoxypropane gave (*S*)-betaxolol in an overall yield of 16%. This approach relies heavily on the use of transition metal catalysts, and the enantiomeric purity comes from the addition of an enantiomerically pure reagent, which is expensive. In 2007, Muthukrishnan et al. prepared enantiopure (*S*)-betaxolol from 4-(2-hydroxyethyl)-phenol in four steps [11]. The first step was the addition of epichlorohydrin to the phenol, and then a kinetic resolution was performed of the resulting epoxide 2-(4-(oxiran-2-ylmethoxy)phenyl)ethan-1-ol using Jacobsen's catalyst. The epoxide was then alkylated and aminated to give (*S*)-betaxolol in 99% *ee* and 33% overall yield. Synthesis of (*S*)-betaxolol in five steps from 4-(2-hydroxyethyl)phenol in 42% overall yield was reported by Zhang et al. in 2009 [12]. This method involves the kinetic resolution of racemic betaxolol with a higher carbon sugar as the chiral auxiliary. The sugar can be synthesized in quite high yield (80%) from 1,4:3,6-dianhydro-D-fructose, which is an expensive sugar of limited availability [13]. The advantage of this method is the use of a non-metallic resolving agent, but the availability of the sugar limits the practical use of this method [12]. Two chemo-enzymatic syntheses of (*S*)-betaxolol have been reported. Di Bono et al. synthesized (*S*)-betaxolol in 91% *ee* by a kinetic resolution of 1-chloro-3-(4-(2-(cyclopropylmethoxy)ethyl)phenoxy)pro-pan-2-ol catalyzed by lipase AK from *Pseudomonas* sp. [14]. The chlorohydrin (*R*)-1-chloro-3-(4-(2-(cyclopropylmethoxy)ethyl)phenoxy)propan-2-ol was isolated in 91% *ee* after the enzymatic reaction, which was converted to (*S*)-betaxolol in 82% *ee* after amination with isopropylamine. The authors report an increase of the *ee* back to 91% upon crystallization of the hydrochloride salt of the drug. In 2011, Li et al. reported a synthesis of (*S*)-betaxolol in 95% *ee* [15]. Their method was based on a kinetic resolution of 4-(2-acetoxy-3-(N-isopropylacetamido)propoxy)phenethyl acetate using the bacterial strain *Rhodotorula mucilaginosa* DQ832198, which was screened from soil. Even though the authors discovered that the selectivity of the lipases from the bacteria was not ideal, giving a maximum of 95% *ee* of the drug, they reported an increase in enantiomeric excess to 99% *ee* after recrystallization of the hydrochloride salt (*S*)-betaxolol hydrochloride.

We have previously reported protocols for obtaining high enantiomeric excess of similar β-blockers as (*S*)-betaxolol with lipase B from *Candida antarctica* (CALB) as catalyst in the kinetic resolution of the secondary halohydrin building blocks [16–18]. Several studies of the active site in CALB based on modelling and experimental data of similar substrates has been published, mostly with an unsubstituted benzene ring coupled to the oxygen moiety in 1-O-alkyl-2-alkanols. A model of the stereoselectivity pocket in CALB is shown in Figure 2 with a general 1-O-alkyl-2-alkanol substrate. The modelling reveals that the -CH_2R_1-group of the faster-reacting enantiomer is located in the stereospecificity pocket and furthermore, that a tryptophane residue (Trp104) limits the size of this pocket [19–21].

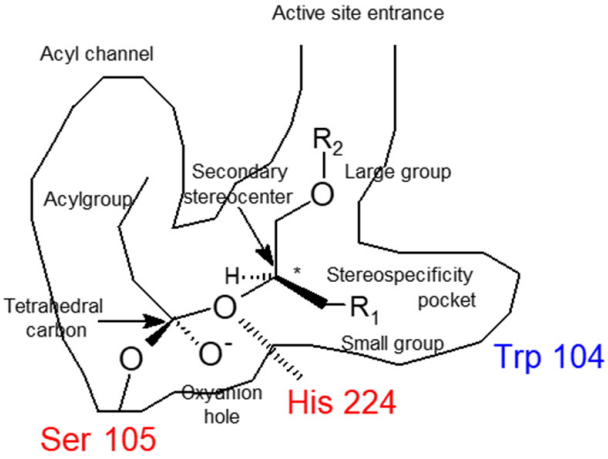


Figure 2. Suggested model for the faster-reacting enantiomer, the *R*-form, of the general substrate 1-O-alkyl-2-alkanol, in the active site of CALB [19].

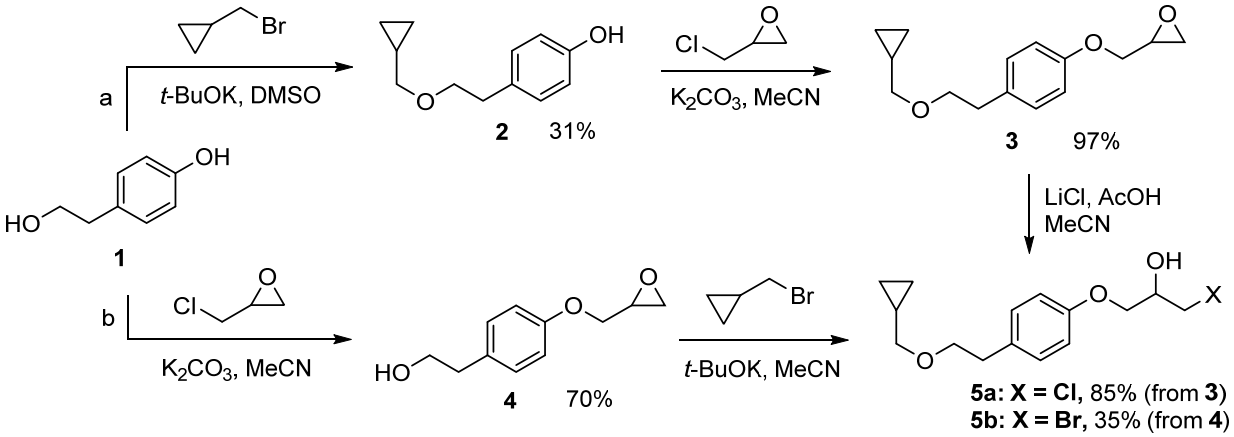
Figure 2 shows a suggested model of the faster-reacting enantiomer, the *R*-form, of the general substrate 1-O-alkyl-2-alkanol, as the tetrahedral intermediate of the butanoates of 1-O-alkyl-2-alkanols from a kinetic resolution of the racemic substrate in the active site of CALB. The large group (-CH_2OR_2) connected to the stereocenter is pointing out of the active site, the acyl group is bound to Ser105, and the small group/halogen (-CH_2R_1) is located in the stereospecificity pocket, which has a tryptophane residue (Trp104) at the bottom. This cavity limits the size of the small group of the substrate [19–21].

We here present a green and sustainable method for (*S*)-betaxolol using a similar synthesis protocol as previously reported with CALB as the catalyst [16–18].

2. Results and Discussion

*2.1. Synthesis of 1-chloro-3-(4-(2-(cyclopropylmethoxy)ethyl)phenoxy)propan-2-ol (**5a**) and 1-bromo-3-(4-(2-(cyclopropylmethoxy)ethyl)phenoxy)propan-2-ol (**5b**)*

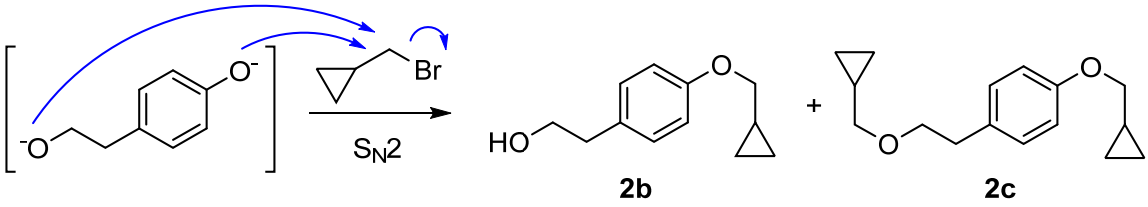
The halohydrins 1-chloro-3-(4-(2-(cyclopropylmethoxy)ethyl)phenoxy)propan-2-ol (**5a**) and 1-bromo-3-(4-(2-(cyclopropylmethoxy)ethyl)phenoxy)propan-2-ol (**5b**) are precursors in the synthesis of betaxolol (**7**). The two halohydrins were synthesized using two different routes from the starting material 4-(2-hydroxyethyl)phenol (**1**), one with the intermediates 4-(2-(cyclopropylmethoxy)ethyl)phenol (**2**) and 2-((4-(2-(cyclopropylmethoxy)ethyl)-phenoxy)-methyl)oxirane (**3**) (Scheme 1a) and the other with the intermediate 2-(4-(oxiran-2-ylmethoxy)phenyl)ethan-1-ol (**4**) (Scheme 1b).



Scheme 1. Synthetic routes to racemic chlorohydrin **5a** (path (**a**)) and bromohydrin **5b** (path (**b**)) from 4-(2-hydroxyethyl)phenol (**1**).

2.1.1. Synthesis of 1-chloro-3-(4-(2-(cyclopropylmethoxy)ethyl)phenoxy)propan-2-ol (**5a**)

Chlorohydrin **5a** was synthesized from **1** in three steps. The intermediate phenol **2** was obtained via a direct alkylation of **1** as reported by Wang et al. [9], by adding (bromomethyl)cyclopropane in the presence of potassium *tert*-butoxide at 50 °C. The strong base generates a dianion where both the phenol group and hydroxyl group of **1** are deprotonated. After 1 h, TLC confirmed full conversion of the starting material, and after purification by flash chromatography, phenol **2** was obtained in 95% purity and 31% yield. As the deprotonated phenoxy group in **1** was not protected, this alkylation step was expected to give by-products resulting from alkylation of the phenoxy group. The by-products were identified by ^{1}H NMR as 2-(4-(cyclopropylmethoxy)phenyl)ethan-1-ol (**2b**) and 1-(cyclopropylmethoxy)-4-(2-(cyclopropylmethoxy)ethyl)benzene (**2c**), as shown in Scheme 2.



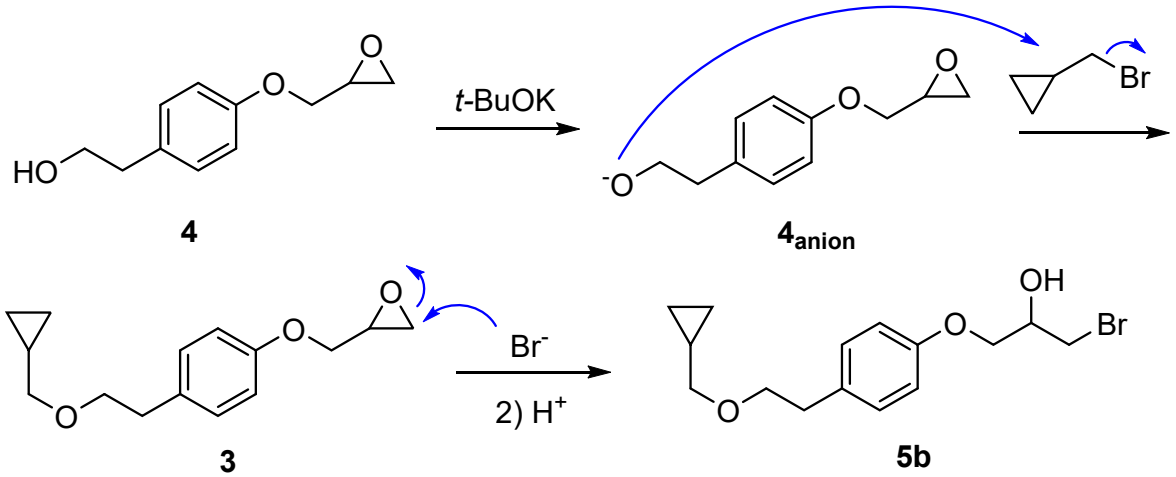
Scheme 2. Mechanism for formation of by-products **2b** and **2c** from the dianion of starting material **1**.

To avoid formation of these by-products the phenolic hydroxy group should be protected, which will increase the yield in this reaction step. Joshi et al. have performed this with a benzyl group [8]. We adapted a new protection method introduced by Brittain et al., which involves protection with a tetrafluoropyridin group [22]. However, we discovered that the tetrafluoropyridin group was incompatible with the strong base required to deprotonate the hydroxyl group. As our focus lies on the enzymatic step, we decided to use the direct alkylation approach. Phenol **2** was converted to epoxide **3** in 97% yield by reaction with epichlorohydrin with potassium carbonate as the base. We have previously described the mechanism for this reaction [17]. Finally, a ring-opening of epoxide **3** using lithium chloride and acetic acid produced the chlorohydrin **5a** in 97% purity and 85% yield. As this reaction proceeded under acidic conditions, the phenolic anion would cause nucleophilic attack on the more substituted carbon of epoxide **3** through a carbocation intermediate. However, we have only seen attack on the less-substituted carbon of epoxide **3** and similar epoxides [16–18]. This indicates that the reaction does not proceed through a carbocation intermediate. The only method that has been previously reported for the synthesis of **5a** was published by Di Bono et al., where the chlorohydrin **5a** was obtained in 80% yield using concentrated hydrochloric acid in chloroform [14].

2.1.2. Synthesis of 1-bromo-3-(4-(2-(cyclopropylmethoxy)ethyl)phenoxy)propan-2-ol (**5b**)

The alternative synthesis approach for bromohydrin **5b** (path b in Scheme 1), is based on reaction of the starting material **1** with epichlorohydrin before alkylation of the primary hydroxy group. This method was adapted from Muthukrishnan et al. and Banoth and Banerjee [11,23]. Phenol **1** was reacted with 3 equivalents of epichlorohydrin in the presence of potassium carbonate in acetonitrile to give epoxide **4** in 70% yield. When 1.1 equivalents of epichlorohydrin were used, the isolated yield of epoxide **4** was lowered to 41%, due to excessive formation of the dimer 2,2′-(((2-hydroxypropane-1,3-diyl)bis(oxy))bis(4,1-phenylene))bis(ethan-1-ol), so we need to add 3 equivalents of epichlorohydrin in this reaction. We have discussed the formation of such dimers in syntheses of similar enantiopure β-blockers previously [18]. Bromohydrin **5b** was obtained in 35% yield by the alkylation of **4** using (bromomethyl)cyclopropane in the presence of potassium *tert*-butoxide. Muthukrishnan et al. used a similar approach in the synthesis of epoxide **3** from **4** [**11**]. In our case, the reaction was allowed to proceed over night at room temperature, which instead led to the formation of bromohydrin **5b**, possibly through a nucleophilic attack of a bromide

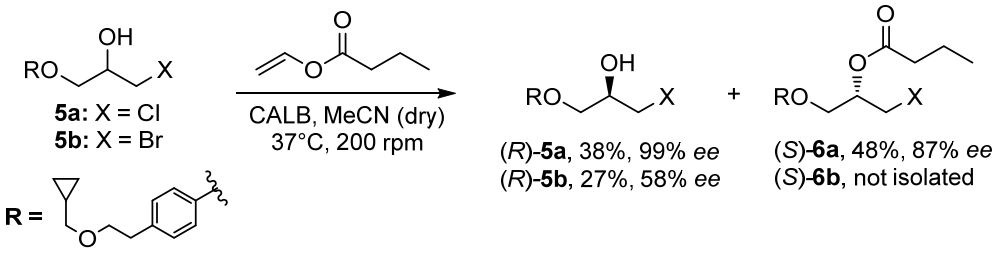
ion on epoxide **3**. Washing the resulting anion with acid could give **5b**. We suggest the mechanism for this transformation in Scheme 3.



Scheme 3. Suggested mechanism for formation of bromohydrin **5b** via a nucleophilic attack of the bromide ion on epoxide **3**.

*2.2. CALB Catalyzed Kinetic Resolution of 1-chloro-3-(4-(2-(cyclopropylmethoxy)ethyl)phenoxy)propan-2-ol (**5a**) and 1-bromo-3-(4-(2-(cyclopropylmethoxy)ethyl)phenoxy)propan-2-ol (**5b**)*

Enzymatic transesterification of the halohydrins **5a** and **5b** was carried out using lipase B from *Candida antarctica* (CALB) in dry acetonitrile with vinyl butanoate as the acyl donor (Scheme 4). CALB has previously been reported to be an effective biocatalyst in the kinetic resolutions of chlorohydrins used in the synthesis of several other β-blockers [16–18]. In the transesterification reactions of **5a** and **5b**, the esters 1-chloro-3-(4-(2-(cyclopropylmethoxy)ethyl)phenoxy)propan-2-yl butanoate (*S*)-**6a** and the equivalent bromo-derivative (*S*)-**6b** were produced, respectively, and chlorohydrins (*R*)-**5a** and (*R*)-**5b** were the remaining unreacted substrates; see Scheme 4.



Scheme 4. CALB-catalyzed transesterification reactions of **5a** with vinyl butanoate as the acyl donor and acetonitrile as the solvent gave (*R*)-**5a** in 99% *ee*. (*R*)-**5b** was obtained in 58% *ee*. The product ester (*S*)-**6a** was isolated with 87% *ee*, whereas (*S*)-**6b** was not isolated.

(*R*)-**5a** was obtained in 99% purity (^{1}H-NMR) and 99% *ee* (chiral HPLC), with a specific rotation of $[\alpha]_D^{20} = -1.92$ (c 1.04, $CHCl_3$), the yield was 38%. The specific rotation of (*R*)-**5a** in 91% *ee* has previously been measured to $[\alpha]_D^{20} + 16$ (c 1, $CHCl_3$) by Di Bono et al. [14]. This is the opposite sign of optical rotation compared to our observation. However, since our measurement of the optical rotation of (*S*)-betaxolol ((*S*)-**7**) agrees with that reported of Di Bono et al. for (*S*)-**7** (see below), we argue that both reported values account for the same enantiomer (the *R*-enantiomer) of chlorohydrin **5a**. The butanoic ester product (*S*)-**6a** was obtained in 96% purity and 87% *ee*, 48% yield, specific rotation $[\alpha]_D^{20} = +13.4$ (c 0.97, $CHCl_3$), which has not been reported previously. In light of the quite similar values for our (*S*)-**6a** ester product, the reported specific rotation of Di Bonos (*R*)-**6** (halohydrin) may instead be of their corresponding ester product. The difference in *ee* in the two reported

halohydrins (our (*R*)-**5a** (99% ee and their (*R*)-**6** (91% ee) should not give such a difference in specific rotation.

The transesterification reaction of bromohydrin **5b** with CALB in acetonitrile gave (*R*)-**5b** in 58% *ee* and 27% yield, much lower than for the **5a**. The difference may be due to the larger size of the bromine substituent compared to the chlorine substituent. A smaller halogen, such as the small group of a halohydrin substrate, will fit more easily in the stereospecificity pocket in the active site of CALB. A bromine atom as the small group in **5b** may also cause electrostatic interactions with the Trp 104 in the stereoselectivity pocket, which will lead to a decrease in the stereoselectivity for the enantiomers allowed in the active site, as we have reported previously [21]. Our results from the synthesis of (*S*)-betaxolol further demonstrate this observation.

The enzymatic kinetic resolution of **5a** was monitored over time to determine the time at which optimal yield and *ee* would be obtained. Samples of 100 µL were withdrawn from the reaction at regular time intervals over a total of 14 h. The samples were analyzed by chiral HPLC to determine the *ee* of the ester product and chlorohydrin reactant. Calculation of the enantiomeric ratio *E* was performed by the software "E&K calculator 2.1b0PPC" [24]. Figure 3 shows the *ee* of the chlorohydrin (*R*)-**5a** (blue squares) and the product ester (*S*)-**6a** (red circles) as functions of conversion. The enantiomeric ratio was determined to be $E = 67$. After 14 h, an *ee* of 99% for **5a** was obtained at 53% conversion. For bromohydrin **5b**, the *E*-value was not determined, as the *ee* did not exceed 58% even after several days of reaction time.

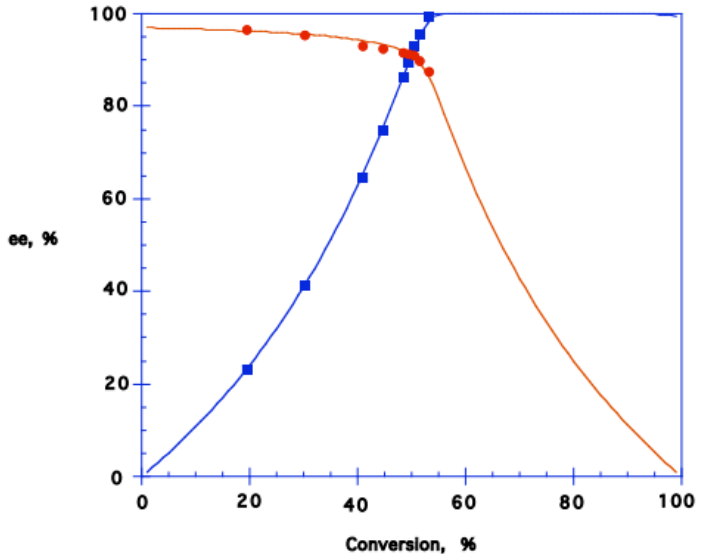
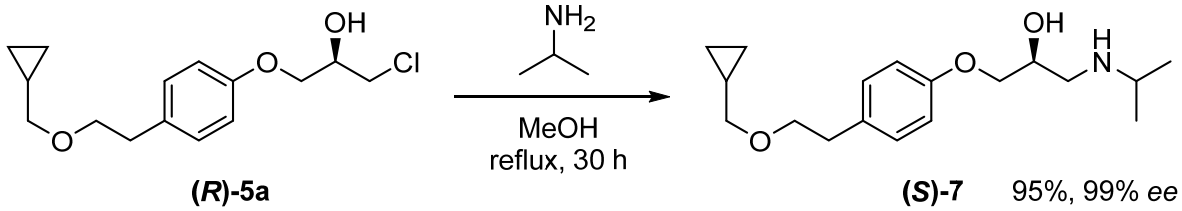


Figure 3. Enantiomeric excess *ee* (%) of chlorohydrin (*R*)-**5a** (blue squares) and the product ester (*S*)-**6a** (red circles) as functions of conversion (%). The enantiomeric ratio was determined to be $E = 67$ ("E&K calculator 2.1b0PPC" [24]). After 14 h, an *ee* of 99% for **5a** was obtained at 53% conversion.

2.3. Synthesis of (S)-betaxolol ((S)-7)

Enantiopure (*S*)-betaxolol ((*S*)-**7**) was obtained via the amination of (*R*)-**5a** with isopropylamine in methanol, according to Scheme 5. The *ee* of the *R*-chlorohydrin was retained in the product. (*S*)-Betaxolol ((*S*)-**7**) was obtained with 98% purity, 99% *ee* in 95% yield. The specific rotation was $[\alpha]_D^{20} = -7.21$ (c 0.97, $CHCl_3$). This value corresponds to the previously reported value of $[\alpha]_D^{20} = -7.13$ (c 1, $CHCl_3$) for (*S*)-**7** in 99% *ee* [8]. See Table 1.



Scheme 5. Synthesis of (*S*)-betaxolol ((*S*)-**7**) in 95% yield and 99% *ee* by amination of chlorohydrin (*R*)-**5a** (99% *ee*) with isopropylamine in methanol.

Table 1. *E*-values, *ee*-values and specific rotations of the enantiopure chlorohydrins (*R*)-**5a** and (*R*)-**5b**, ester (*S*)-**6a** and the drug (*S*)-**7**. Specific rotations were determined at 20 °C in $CHCl_3$. For additional parameters, see Section 3. For spectra and chromatograms, see Supplementary Materials.

Enantiomer	*E*-Value	Specific Rotation, *ee* %	Literature Value
(*R*)-**5a**	57	$[\alpha]_D^{20} = -1.92$ (*c* 1.04, $CHCl_3$), 99% *ee*	$[\alpha]_D^{20} = +16.00$ (*c* 1.0, $CHCl_3$), 91% *ee* [14]
(*R*)-**5b**	-		
(*S*)-**6a**	57	$[\alpha]_D^{20} = +13.40$ (*c* 0.97, $CHCl_3$), 87 % *ee*	
(*S*)-**7**	-	$[\alpha]_D^{20} = -7.21$ (*c* 0.97, $CHCl_3$), 99% *ee*	$[\alpha]_D^{20} = -7.13$ (*c* 1.0, $CHCl_3$), 99% *ee* [8]

3. Materials and Methods

3.1. Chemicals and Solvents

Chemicals and solvents were purchased from Sigma Aldrich (Oslo, Norway). Distilled water was obtained from an ELGA PURELAB flex purification system. Dry MeCN was obtained from an MBraun MB-SPS-800 solvent purifier (München, Germany), and stored over 4 Å molecular sieves. Molecular sieves were dried at 1000 °C for 24 h and stored in a dessicator.

3.2. TLC-Analyses and Column Chromatography

TLC-analyses were performed on Merck silica 60 F_{254} and detected with UV light at 254 nm. Flash chromatography was performed using silica gel from Sigma-Aldrich Norway (Oslo, Norway) (pore size 60 Å, 230–400 mesh particle size, 40–63 µm particle size).

3.3. Enzyme Preparations

Candida antarctica lipase B (CALB) expressed in *Pichia pastoris* and immobilized on a hydrophobic polymer resin (>10,000 PLU, lot# 20170315) was kindly gifted from SyncoZymes Co., Ltd. (Shanghai, China).

Enzymatic reactions were carried out in a New Brunswick G24 Environmental Incubator Shaker (Edison, NJ, USA) at 37 °C and 200 rpm.

3.4. NMR Analyses

NMR-analyses were recorded on a Bruker 400 MHz Avance III HD instrument equipped with a 5 mm SmartProbe Z-gradient probe operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C, respectively, or on a Bruker 600 MHz Avance III HD instrument equipped with a 5 mm cryogenic CP-TCI Z-gradient probe operating at 600 MHz for ^{1}H and 150 MHz for ^{13}C, COSY, HSQC and HMBC. NMR spectra are included in the Supplementary Materials for relevant compounds. (Bruker, Rheinstetten, Germany). Chemical shifts are in ppm relative to TMS (or $CHCl_3$ shift) and coupling constants are in hertz (Hz). ^{1}H- and ^{13}C NMR spectra can be found in the Supplementary Materials.

3.5. Optical Rotation

Optical rotation values were performed with an Anton Paar MCP 5100 polarimeter from Dipl. Ing. Houm AS (Oslo, Norway), wavelength of 589 nm (D) and a path length of 10 mm. See single enantiomers for specific rotation values.

3.6. Chiral HPLC Analyses

Chiral HPLC analyses were performed on an Agilent 1200 HPLC system equipped with an autosampler (10 µL injection volume) and a diode array detector (DAD) and a variable wavelength detector (VWD) set to 254 nm) (Agilent Technologies, Palo Alto, CA, USA). Separation of enantiomers were performed on a Daicel Chiralcel OD-H column (cellulose tris (3,5-dimethylphenylcarbamate) on silica gel, 250 mm × 4.6 mm ID, 5 µm particle size) (Daicel, Chiral Technologies Europe, Gonthier d'Andernach, Illkirch, France). Separation of **3**, **5a** and **5b**: *n*-hexane:*i*-PrOH (90:10) flow rate 1 mL min^{-1}. t_R(**3**) = 7.1 min, t_R(**3**) = 8.4 min, t_R((*S*)-**5a**) = 10.8 min, t_R((*R*)-**5a**) = 12.8 min, t_R((*S*)-**5b**) = 11.3 min, t_R((*R*)-**5b**) = 13.7 min. Separation of **6a**: *n*-hexane:*i*-PrOH (99:1) flow rate 1 mL min^{-1}. t_R((*S*)-**6a**) = 14.4 min, t_R((*R*)-**6a**) = 15.7 min. Separation of **7**: *n*-hexane:*i*-PrOH:diethylamine (90:9.8:0.2), flow rate: 1 mL min^{-1}. t_R((*R*)-**7**) = 6.4 min, t_R((*S*)-**7**) = 12.4 min.

3.7. Liquid Chromatography-Mass Spectroscopy (LC-MS)

LC-MS analyses were performed on an AQUITY UPLC I-Class system (Waters Corporation, Milford, MA, USA) coupled to a quadruple time-of-flight mass analyzer (QTOF; SYNAPT-G2S) with a ZSpray EIS ion source (Waters, Milford, USA) with an ACQUITY UPLC HSS T3 column (100 mm × 2.1 mm ID, 100 Å × 1.8 µm film thickness) with a mobile phase of A: water with 0.12% NH_4OH and B: MeCN, flow rate: 0.25 mL min^{-1}. The analyses were run with a gradient of 10–100% B for 12 min, 2 min hold, then 100–10% B for 1 min. Total time, 15.0 min.

3.8. Mass Spectrometry Analysis

Exact masses were determined with a Synapt G2-S Q-TOF mass spectrometer from Waters^{TM} (Waters Norway, Oslo, Norway). Ionization of sample was conducted with an ASAP probe (APCI), and calculation of exact masses and spectra processing were performed with Waters^{TM} Software Masslynxs Version 4.1, SCN871, Waters Corporation (Milford, MA, USA). See Supplementary Materials for spectra.

3.9. Synthesis Protocols

3.9.1. 4-(2-(Cyclopropylmethoxy)ethyl)phenol (**2**)

A solution of 4-(2-hydroxyethyl)phenol (**1**) (1.01 g, 7.24 mmol) and *t*-BuOK (2.44 g, 21.7 mmol) in DMSO (5 mL) was stirred at 50 °C for 30 min under N_2 atmosphere. A solution of (bromomethyl)cyclopropane (1.10 mL, 10.8 mmol) in DMSO (5 mL) was then added dropwise, and the reaction was stirred for 1 h under the same conditions. The reaction was cooled to rt and quenched with water (10 mL), then washed with toluene (2 × 5 mL) to remove impurities. The aqueous phase was added to dilute HCl until it reached neutral pH and extracted with toluene (4 × 15 mL). The combined organic phase was washed with water (5 × 20 mL) to remove excess DMSO, and dried over $MgSO_4$. The solvent was removed under reduced pressure. The crude mixture (0.64 g) was purified by flash chromatography (1:11, MeCN:CH_2Cl_2) to afford 4-(2-(cyclopropylmethoxy)ethyl)phenol (**2**) as a clear oil with 95% purity (^{1}H NMR) in 31% yield (0.42 g, 2.21 mmol). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.06–7.09 (m, 2H, Ar-**H**), 6.73–6.75 (m, 2H, Ar-**H**), 5.37 (s, 1H, Ar-O**H**), 3.65 (t, 2H, ^3J = 7.43 Hz, CH_2CH_2O), 3.30 (d, 2H, ^3J = 7.00 Hz, CH_2O-CH), 2.84 (t, 2H, ^3J = 7.43 Hz, Ar-CH_2), 1.03–1.10 (m, 1H, C**H**), 0.51–0.55 (m, 2H, CH_2), 0.19–0.22 (m, 2H, CH_2). ^{13}C NMR (150 MHz, $CDCl_3$) δ 154.3, 130.8, 130.0, 115.4, 75.8, 72.0, 35.5, 10.7, 3.2.

3.9.2. 2-((4-(2-(Cyclopropylmethoxy)ethyl)phenoxy)methyl)oxirane (**3**)

To a stirred solution of 4-(2-(cyclopropylmethoxy)ethyl)phenol (**2**) (0.20 g, 1.04 mmol) and K_2CO_3 (0.43 g, 3.12 mmol) in dry MeCN (15 mL), epichlorohydrin (0.33 mL, 4.16 mmol) was added. The mixture was heated under reflux for 22 h. Full conversion was confirmed by TLC (1:11, MeCN:CH_2Cl_2); R_f (**2**) = 0.48, R_f (**3**) = 0.61. The mixture was filtered, and the filtrate was concentrated under reduced pressure. EtOAc (25 mL) was added, and the solution was washed with distilled water. The aqueous phase was extracted with

EtOAc (3 × 15 mL), and the combined organic phase was washed with brine and dried over $MgSO_4$. Epoxide **3** was obtained as a light-yellow liquid with 98% purity (^{1}H NMR) in 97% yield (0.25 g, 1.01 mmol). HPLC eluent: *n*-hexane:*i*-PrOH (90:10), t_R(**3**) = 7.1 min, t_R(**3**) = 8.4 min. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.13–7.15 (m, 2H, Ar-**H**), 6.83–6.86 (m, 2H, Ar-**H**), 4.18 (dd, 1H, ^3J = 3.35 Hz, ^2J = 11.13 Hz, O-C**H**$_2$CH-O), 3.93 (dd, 1H, ^3J = 5.40 Hz, ^2J = 11.13 Hz, O-C**H**$_2$CH-O), 3.61 (t, 2H, ^3J = 7.60 Hz, O-C**H**$_2$CH$_2$), 3.32–3.35 (m, 1H, C**H**-O), 3.27 (d, 2H, ^3J = 6.71 Hz, C**H**$_2$-O), 2.89 (t, 1H,^3J = 4.76 Hz, ^2J = 4.76 Hz, C**H**$_2$ (epoxide)), 2.84 (t, 2H, ^3J = 7.60 Hz, C**H**$_2$-Ar), 2.74 (dd, 1H, ^2J = 4.76 Hz, ^3J = 2.70 Hz, C**H**$_2$ (epoxide)), 1.02–1.08 (m, 1H, C**H** (cyclopropyl)), 0.51–0.54 (m, 2H, C**H**$_2$ (cyclopropyl)), 0.18–0.21 (m, 2H, C**H**$_2$ (cyclopropyl)). ^{13}C NMR (150 MHz, $CDCl_3$) δ 157.1, 131.8, 129.9, 114.7, 75.7, 71.9, 68.9, 50.3, 44.8, 35.6, 10.7, 1.3.

3.9.3. 2-(4-(Oxiran-2-ylmethoxy)phenyl)ethan-1-ol (**4**)

To a solution of 4-(2-hydroxyethyl)phenol (**1**) (1.10 g, 7.94 mmol) and K_2CO_3 (3.45 g, 25.0 mmol) in dry MeCN (20 mL), epichlorohydrin (1.70 mL, 21.6 mmol) was added. The mixture was stirred under reflux for 23 h. Full conversion was detected by TLC (1:10, MeOH:CH_2Cl_2); R_f (**4**) = 0.45. The mixture was filtered, and the solvent removed under reduced pressure. The crude mixture (1.31 g) was purified by flash chromatography (1:10, MeOH:CH_2Cl_2) to yield **4** as a white crystalline solid with 99% purity (^{1}H NMR) in 70% yield (1.06 g, 5.46 mmol). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.13–7.15 (m, 2H, Ar-**H**), 6.86–6.88 (m, 2H, Ar-**H**), 4.19 (dd, 1H, ^3J = 3.12 Hz, ^2J = 11.16 Hz, O-C**H**$_2$CH-O), 3.94 (dd, 1H, ^3J = 5.58 Hz, ^2J = 11.16 Hz, O-C**H**$_2$CH-O), 3.80 (q, 2H, ^3J = 6.60 Hz, C**H**$_2$-OH), 3.33–3.35 (m, 1H, C**H**), 2.90 (t, 1H, ^2J = ^3J = 4.70 Hz, C**H**$_2$ (epoxide)), 2.80 (t, 2H, ^3J = 6.29 Hz, C**H**$_2$-Ar), 2.75 (dd, ^2J = 4.70 Hz, ^3J = 2.58 Hz, C**H**$_2$ (epoxide)), 1.52 (t, 1H, ^3J = 5.70 Hz, O**H**). ^{13}C NMR (150 MHz, $CDCl_3$) δ 157.3, 131.2, 114.9, 68.9, 63.9, 50.3, 44.8, 38.4.

3.9.4. 1-Chloro-3-(4-(2-(cyclopropylmethoxy)ethyl)phenoxy)propan-2-ol (**5a**)

To a stirred solution of 2-((4-(2-(cyclopropylmethoxy)ethyl)phenoxy)methyl)oxirane (**3**) (0.22 g, 0.87 mmol) and LiCl (80.0 mg, 1.74 mmol) in MeCN (5 mL), glacial AcOH (250 mL, 4.35 mmol) was added. The solution was stirred at rt for 17 h, monitored by TLC (1:11, MeCN:CH_2Cl_2); R_f (**3**) = 0.61, R_f (**5a**) = 0.45. The reaction was quenched with $NaHCO_3$ (aq) until neutral pH. The aqueous mixture was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phase was washed with brine and dried over $MgSO_4$. The solvent was removed under reduced pressure to give chlorohydrin (**5a**) as a pale-yellow oil in 85% yield (0.23 g, 0.82 mmol) and 97% purity (^{1}H NMR). HPLC eluent: *n*-hexane:*i*-PrOH (90:10), t_R((*S*)-**5a**) = 10.8 min, t_R((*R*)-**5a**) = 12.8 min. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.14–7.16 (m, 2H, Ar-**H**), 6.83–6.86 (m, 2H, Ar-**H**), 4.20 (sx, 1H, ^3J = 5.40 Hz, C**H**-OH), 4.04–4.09 (m, 2H, O-C**H**$_2$-CHOH), 3.70–3.79 (m, 2H, C**H**$_2$-Cl), 3.62 (t, 2H, ^3J = 7.27 Hz, CH$_2$-C**H**$_2$O), 3.28 (d, 2H, ^3J = 6.79 Hz, C**H**$_2$-cyclopropyl)), 2.85 (t, 2H,^3J = 7.27 Hz, Ar-C**H**$_2$), 2.62–2.66 (m, 1H, O**H**), 1.02–1.09 (m, 1H, C**H** (cyclopropyl)), 0.51–0.54 (m, 2H, C**H**$_2$ (cyclopropyl)), 0.18–0.21 (m, 2H, C**H**$_2$ (cyclopropyl)). ^{13}C NMR (150 MHz, $CDCl_3$) δ 156.8, 132.1, 130.1, 114.6, 75.8, 71.8, 70.0, 68.9, 46.1, 35.6, 10.7, 3.1.

3.9.5. 1-Bromo-3-(4-(2-(cyclopropylmethoxy)ethyl)phenoxy)propan-2-ol (**5b**) Correct Procedure

A solution of 2-(4-(oxiran-2-ylmethoxy)phenyl)ethan-1-ol (**4**) (0.58 g, 2.98 mmol) (bromomethyl)cyclopropane (371 µL 3.57 mmol) in MeCN (5 mL) was stirred under N_2 for 15 min. The solution was cooled to 0 °C and *t*-BuOK (0.47 g, 4.17 mmol) was added slowly over a period of 10 min. The mixture was stirred under N_2 at 0 °C for 5 h, then left at rt for 19 h. The reaction was monitored by TLC (1:10, MeOH:CH_2Cl_2); R_f (**4**) = 0.45, R_f (**5b**) = 0.72. The reaction was quenched with aqueous H_2SO_4 (4 M, 3 mL), and extracted with Et_2O (3 × 15 mL). The combined organic phase was washed with water (2 × 10 mL) and brine (10 mL), and dried over $MgSO_4$. The solvent was removed under reduced pressure. The crude mixture (0.89 g) was purified by flash chromatography (1:10, MeOH:CH_2Cl_2) to

yield bromohydrin **5b** as a pale-yellow oil in 35% yield. HPLC eluent: *n*-hexane:*i*-PrOH (90:10), t_R((*S*)-**5b**) = 11.3 min, t_R((*R*)-**5b**) = 13.7 min. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.14–7.16 (m, 2H, Ar-**H**), 6.83–6.86 (m, 2H, Ar-**H**), 4.17–4.20 (m, 1H, C**H**-OH), 4.04–4.10 (m, 2H, O-C$\mathbf{H}_2$-CHOH), 3.58–3.67 (m, 4H, C$\mathbf{H}_2$-Cl, CH_2-C$\mathbf{H}_2$O), 3.28 (d, 2H, ^3J =7.17 Hz, C$\mathbf{H}_2$-cyclopropyl)), 2.85 (t, 2H, ^3J =7.20 Hz, Ar-C$\mathbf{H}_2$), 2.53–2.55 (m, 1H, O**H**), 1.04–1.07 (m, 1H, C**H** (cyclopropyl)), 0.51–0.54 (m, 2H, C$\mathbf{H}_2$ (cyclopropyl)), 0.18–0.21 (m, 2H, C$\mathbf{H}_2$ (cyclopropyl)). ^{13}C NMR (150 MHz, $CDCl_3$) δ 156.8, 132.2, 130.1, 114.6, 75.8, 71.9, 69.7, 69.4, 35.6, 35.2, 10.8, 3.1. MS (TOF MS ES+): m/z = 351.0572 $[M + Na]^+$, (calc. mass $[M + Na]^+$ = 351.06, $C_{15}H_{21}BrNaO_3$).

3.9.6. Synthesis of 1-chloro-3-(4-(2-(cyclopropylmethoxy)ethyl)phenoxy)propan-2-yl butanoate (**6a**)

A small vial was charged with 1-chloro-3-(4-(2-(cyclopropylmethoxy)ethyl)phenoxy)-propan-2-ol (**5a**) (18.0 mg, 0.06 mmol), butanoic acid (2 drops), pyridine (2 drops) and *n*-hexane (0.5 mL). The vial was placed in an air bath at 60 °C for 1 h. The solution was washed with distilled water (5 × 1 mL) and dried over $MgSO_4$. Excess pyridine was removed by adding toluene (3 mL) and evaporating under reduced pressure. This process was repeated before the sample was analyzed by HPLC eluent: *n*-hexane:*i*-PrOH (99:1), t_R((*S*)-**6a**) = 14.4 min, t_R((*R*)-**6a**) = 15.7 min.

3.9.7. Synthesis of Racemic Betaxolol (**7**)

To a stirred solution of 1-chloro-3-(4-(2-(cyclopropylmethoxy)ethyl)phenoxy)propan-2-ol (**5a**) (24.9 mg, 0.09 mmol) in MeOH (2 mL), isopropylamine (157 µL, 1.80 mmol) was added. The solution was stirred under reflux for 22 h. Full conversion was detected by TLC (1:11, MeCN:CH_2Cl_2); R_f (**5a**) = 0.45, R_f (**7**) = 0.05. The solution was diluted with EtOAc (50 mL) and washed with $NaHCO_3$ (aq, 10 mL) and distilled water (2 × 20 mL). The combined organic phase was dried over $MgSO_4$ and concentrated under reduced pressure to yield betaxolol (**7**) with 95% purity as a pale pink oil in 57% yield (15.7 mg, 0.05 mmol). HPLC eluent: *n*-hexane:*i*-PrOH:diethylamine (90:9.8:0.2), t_R((*R*)-**7**) = 6.4 min, t_R((*S*)-**7**) = 12.4 min. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.12–7.14 (m, 2H, Ar-**H**), 6.82–6.84 (m, 2H, Ar-**H**), 4.15–4.18 (m, 1H, C**H**-OH), 3.93–4.00 (m, 2H, C$\mathbf{H}_2$-Ar), 3.81 (bs, 2H, O**H**, N**H**), 3.60 (t, 2H, ^3J = 7.38 Hz, O-C$\mathbf{H}_2$-CH_2), 3.28 (d, 2H, ^3J = 6.85 Hz, O-C$\mathbf{H}_2$-CH-), 2.98–3.01 (m, 2H, C$\mathbf{H}_2$-NH-, C**H**-NH-), 2.84 (t, 2H, ^3J = 7.38 Hz, C$\mathbf{H}_2$-Ar), 2.82–2.85 (m, 1H, C$\mathbf{H}_2$-NH-), 1.19 (dd, 6H, ^3J = 3.17, ^3J = 6.34, C$\mathbf{H}_3$), 1.04–1.06 (m, 1H, C**H** (cyclopropyl)), 0.51–0.54 (m, 2H, C$\mathbf{H}_2$ (cyclopropyl)), 0.18–0.20 (m, 2H, C$\mathbf{H}_2$ (cyclopropyl)). ^{13}C NMR (150 MHz, $CDCl_3$) δ 157.2, 131.7, 130.0, 114.6, 75.8, 71.9, 70.4, 67.9, 49.8, 49.2, 35.6, 22.2, 22.0, 10.8, 3.1.

3.9.8. CALB Catalyzed Kinetic Resolution of 1-chloro-3-(4-(2-(cyclopropylme-thoxy)ethyl)phenoxy)propan-2-ol (**5a**)

To a solution of 1-chloro-3-(4-(2-(cyclopropylmethoxy)ethyl)phenoxy)propan-2-ol (**5a**) (0.17 g, 0.61 mmol) in dry MeCN (12 mL) containing activated 4Å molecular sieves, vinyl butanoate (319 µL, 2.52 mmol) and CALB (360 mg) was added. The reaction vial was capped and placed in an incubator at 37 °C and 200 rpm for 14 h. The reaction mixture was filtered, and the solvent was removed under reduced pressure before separation of (*R*)-1-chloro-3-(4-(2-(cyclopropylme-thoxy)ethyl)phenoxy)propan-2-ol ((*R*)-**5a**) and (*S*)-1-chloro-3-(4-(2-(cyclopropylmethoxy)ethyl)phenoxy)pro-pan-2-yl butanoate ((*S*)-**6a**) by flash chromatography (1:4 EtOAc/*n*-pentane); R_f (**5a**) = 0.28, R_f (**6a**) = 0.63. The two fractions were diluted with EtOAc (15 mL) and washed with distilled water (3 × 10 mL). The combined aqueous phase was extracted with EtOAc (10 mL). The combined organic phase was washed with brine (15 mL), dried over $MgSO_4$ and concentrated under reduced pressure. Chlorohydrin (*R*)-**5a** was isolated as a transparent oil in 38% yield (0.06 g, 0.23 mmol), 99% purity (^{1}H NMR) and 99% *ee* (HPLC), $[\alpha]_D^{20} = -1.92$ (c 1.04, $CHCl_3$). Ester (*S*)-**6a** was isolated as a yellow oil with 96% purity (^{1}H NMR), 87% *ee* in 48% yield (0.10 g, 0.29 mmol). (HPLC), $[\alpha]_D^{20} = +13.4$ (c 0.97, $CHCl_3$), ^{1}H NMR (600 MHz, $CDCl_3$) of **6a**: δ 7.13–7.16 (m, 2H, Ar-**H),** 6.83–6.85 (m, 2H, Ar-**H**), 5.33 (quint, 1H, ^3J = 5.11 Hz, C**H**),

4.12–4.22 (m, 2H, C**H**$_2$-OAr), 3.76–3.86 (m, 2H, CH$_2$-Cl), 3.59–3.62 (m, 2H, ^3J = 7.46 Hz, C**H**$_2$-O), 3.28 (d, 2H, ^3J = 7.16 Hz, CH$_2$-cyclopropyl), 2.83–2.86 (m, 2H, ^3J = 7.46 Hz, C**H**$_2$-Ar), 2.32–2.37 (m, 2H, C**H**$_2$-COOR), 1.65–1.70 (m, 2H, C**H**$_2$CH$_3$), 1.04–1.07 (m, 1H, C**H** (cyclopropyl)), 0.93–0.99 (m, 3H, C**H**$_3$), 0.51–0.54 (m, 2H, C**H**$_2$ (cyclopropyl)), 0.18–0.21 (m, 2H, C**H**$_2$ (cyclopropyl)). ^{13}C NMR (150 MHz, CDCl$_3$) of **9**: δ 172.9, 156.9, 132.1, 130.1, 114.7, 75.8, 71.9, 71.0, 66.3, 42.8, 36.2, 35.6, 18.6, 13.7, 10.7, 3.1.

3.9.9. CALB catalyzed Kinetic Resolution of 1-bromo-3-(4-(2-(cyclopropylme-thoxy)ethyl)phenoxy)propan-2-ol (**5b**)

To a solution of 1-bromo-3-(4-(2-(cyclopropylmethoxy)ethyl)phenoxy)propan-2-ol (**5b**) (0.11 g, 0.33 mmol) in dry MeCN (12 mL) containing activated 4Å molecular sieves, vinyl butanoate (166 µL, 1.32 mmol) and CALB (230 mg) was added. The reaction vial was capped and placed in an incubator at 37 °C and 200 rpm for 14 h. The enzyme was filtered off and the solvent was removed under reduced pressure before separation of (*R*)-1-bromo-3-(4-(2-(cyclopropylme-thoxy)ethyl)phenoxy)propan-2-ol ((*R*)-**5b**) and (*S*)-1-bromo-3-(4-(2-(cyclopropylmethoxy)ethyl)phenoxy)pro-pan-2-yl butanoate ((*S*)-**6b**) by flash chromatography (1:4, EtOAc:*n*-pentane); R_f (**5b**) = 0.29, R_f (**6b**) = 0.50. The fraction containing (*R*)-**5b** was diluted with EtOAc (15 mL) and washed with distilled water (15 mL) and brine (10 mL), dried over MgSO$_4$ and concentrated under reduced pressure. Bromohydrin (*R*)-**5b** was isolated as a light-yellow oil in 27% yield (30.0 mg, 0.09 mmol) and 58% *ee*.

3.9.10. Synthesis of (*S*)-betaxolol ((*S*)-**7**) from (*R*)-**5a**

Following the procedure described in Section 3.9.7 (*R*)-**5a** (33.0 mg, 0.12 mmol, 99% *ee)* was converted to (*S*)-betaxolol ((*S*)-**7)** appearing as a pale-pink oil in 95% (step) yield (33.8 mg, 0.11 mmol), 98% purity (^{1}H NMR) and 99% *ee* (HPLC), $[\alpha]_D^{20}$= −7.21 (*c* 0.97, CHCl$_3$).

4. Conclusions

The enantiopure chlorohydrin (*R*)-**5a** was produced in 99% *ee* and 38% yield via a CALB catalyzed kinetic resolution of **5a**. The amination reaction (*R*)-**5a** gave (*S*)-betaxolol ((*S*)-**7**) in 95% yield and 99% *ee*. The overall yield from the starting phenol **1** to (*S*)-**7** was 9%. This protocol for (*S*)-betaxolol is considered as an environmentally friendly alternative compared to previously reported methods, however the total yield can be improved. A dispute of the previously reported optical rotations of chlorohydrin (*R*)-**5a** and the product ester (*S*)-**6a** from the kinetic resolution of **5a** by Di Bono [14] has been introduced. The specific rotation of (*R*)-**5a** (99% *ee*) was determined to be $[\alpha]_D^{20}$ = −1.92 (*c* 1.04, CHCl$_3$) by us, and for (*S*)-**6a** (87% *ee)* it was determined to be $[\alpha]_D^{20}$ = +13.4 (*c* 0.97, CHCl$_3$). Di Bono et al. report a specific rotation of the same compound as our (*R*)-**5a** in 91% *ee* [their (*R*)-**6a**] to $[\alpha]_D^{20}$ + 16.00 (c 1, CHCl$_3$). We suppose that this must be due to a confusion of the enantiomers measured from their side, since they conclude that their (−)-**6** is converted to (*S*)-betaxolol.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/catal12121645/s1, ^{1}H and ^{13}C NMR spectra, MS spectra and relevant chiral HPLC chromatograms.

Author Contributions: Investigation, writing, original draft preparation, S.H.T.; supervision and writing, review and editing, E.E.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The data presented in this study are available online.

Acknowledgments: EEA project 18-COP-0041 GreenCAM is thanked for their support; SyncoZymes CoLTD, Shanghai, China is thanked for the gift of CALB.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Al-wadei, M.J.; Bakheit, A.H.; Abdel-Aziz, A.A.; Wani, T.A. *Chapter Three-Betaxolol: A Comprehensive Profile in Profiles of Drug Substances, Excipients and Related Methodology*; Al-Majed, A., Ed.; Academic Press: Cambridge, MA, USA, 2021; pp. 91–136, ISBN 18715125. [CrossRef]
2. Weinreb, R.N.; Khaw, P.T. Primary open-angle glaucoma. *Lancet* **2004**, *363*, 1711–1720. [CrossRef] [PubMed]
3. Quigley, H.A.; Broman, A.T. The number of people with glaucoma worldwide in 2010 and 2020. *Br. J. Ophthalmol.* **2006**, *90*, 262–267. [CrossRef] [PubMed]
4. Agustian, J.; Kamaruddin, A.; Bhatia, S. Single enantiomeric b-blockers. The existing technologies. *Process Biochem.* **2010**, *45*, 1587–1604. [CrossRef]
5. FDA's policy statement for the development of new stereoisomeric drugs. *Chirality* **1992**, *4*, 338–340. [CrossRef]
6. Calcaterra, A.; D'Acquarica, I. The market of chiral drugs: Chiral switches versus de novo enantiomerically pure compounds. *J. Pharm. Biomed. Anal.* **2018**, *147*, 323–340. [CrossRef]
7. Manoury, P.M.; Binet, J.L.; Rousseau, J.; Lefevre-Borg, F.M.; Cavero, I.G. Synthesis of a series of compounds related to betaxolol, a new b_1-adrenoceptor antagonist with a pharmacological and pharmacokinetic profile optimized for the treatment of chronic cardiovascular diseases. *J. Med. Chem.* **1987**, *30*, 1003–1011. [CrossRef]
8. Joshi, R.A.; Garud, D.R.; Muthukrishnan, M.; Joshi, R.R.; Gurjar, M.K. A convenient synthesis of the enantiomerically pure b-blocker (*S*)-betaxolol using hydrolytic kinetic resolution. *Tetrahedron: Asymmetry* **2005**, *16*, 3802–3806. [CrossRef]
9. Wang, X.W.; Bhatia, A.V.; Chamberlin, S.A.; Luping, L. Selective alkylation of an alcohol substituted phenol compound. U.S. Patent 5731463, 24 March 1998.
10. Datta, G.K.; von Schenck, H.; Hallberg, A.; Larhed, M. Selective Terminal Heck Arylation of Vinyl Ethers with Aryl Chlorides, A Combined Experimental Computational Approach Including Synthesis of Betaxolol. *J. Org. Chem.* **2006**, *71*, 3896–3903. [CrossRef]
11. Muthukrishnan, M.; Garud, D.R.; Joshi, R.R.; Joshi, R.A. Concise synthesis of b-blockers (*S*)-metoprolol and (*S*)-betaxolol using hydrolytic kinetic resolution. *Tetrahedron* **2007**, *63*, 1872–1876. [CrossRef]
12. Zhang, J.-Y.; Liu, H.-M.; Wang, X.-J.; Wang, P.; Zheng, J.-X. Application of kinetic resolution using HCS as chiral auxiliary, Novel synthesis of b-blockers (*S*)-betaxolol and (S)-metoprolol. *Chirality* **2009**, *21*, 745–750. [CrossRef]
13. Liu, H.-M.; Liu, F.-W.; Song, X.-P.; Zhang, J.-Y.; Yan, L. A novel free C-12 higher carbon sugar: Asymmetric synthesis and reactivity with nucleophiles. *Tetrahedron: Asymmetry* **2006**, *17*, 3230–3236. [CrossRef]
14. Di Bono, G.; Scilimati, A. A Chemoenzymatic Route to Both Enantiomers of Betaxolol. *Synthesis* **1995**, *6*, 699–702. [CrossRef]
15. Li, Y.-H.; Huang, L.-H.; Liu, H.-M. Chemoenzymatic Route to *S*-Betaxolol. *Synth. Commun.* **2011**, *41*, 2468–2474. [CrossRef]
16. Lund, I.T.; Bøckmann, P.L.; Jacobsen, E.E. Highly enantioselective CALB catalyzed kinetic resolution of building blocks for b-blocker atenolol. *Tetrahedron* **2016**, *72*, 7288–7292. [CrossRef]
17. Gundersen, M.A.; Austli, G.B.; Løvland, S.S.; Hansen, M.B.; Rødseth, M.; Jacobsen, E.E. Lipase Catalyzed Synthesis of Enantiopure Precursors and Derivatives for β-Blockers Practolol, Pindolol and Carteolol. *Catalysts* **2021**, *11*, 503. [CrossRef]
18. Troøyen, S.H.; Bocquin, L.; Tennfjord, A.L.; Klungseth, K.; Jacobsen, E.E. Green Chemo-Enzymatic Protocols for the Synthesis of Enantiopure b-Blockers (*S*)-Esmolol and (*S*)-Penbutolol. *Catalysts* **2022**, *12*, 980. [CrossRef]
19. Uppenberg, J.; Öhrner, N.; Norin, M.; Hult, K.; Kleywegt, G.J.; Patkar, S.; Waagen, V.; Anthonsen, T.; Jones, T.A. Crystallographic and molecular modeling studies of lipase B from *Candida antarctica* reveal a stereospecificity pocket for secondary alcohols. *Biochemistry* **1995**, *34*, 16838–16851. [CrossRef]
20. Jacobsen, E.E.; Hoff, B.H.; Anthonsen, T. Enantiopure derivatives of 1,2-alkanediols. Substrate requirements for lipase B fromCandida antarctica. *Chirality* **2000**, *12*, 654–659. [CrossRef]
21. Jacobsen, E.E.; Anthonsen, T. Water content influences the selectivity of CALB-catalyzed kinetic resolution of phenoxymethyl-substituted secondary alcohols. *Can. J. Chem.* **2002**, *80*, 577–581. [CrossRef]
22. Brittain, W.D.G.; Cobb, S.L. Tetrafluuoropyridyl (TFP): A general phenol protecting group readily cleaved under mild conditions. *Org. Biomol. Chem.* **2019**, *17*, 2110–2115. [CrossRef]
23. Banoth, L.; Banerjee, U.C. New chemical and chemo-enzymatic synthesis of (*RS*)-, (*R*)-, and (*S*)-esmolol. *Arab. J. Chem.* **2017**, *10*, S3603–S3613. [CrossRef]
24. Anthonsen, H.W.; Hoff, B.H.; Anthonsen, T. Calculation of enantiomer ratio and equilibrium constants in biocatalytic ping-pong bi-bi resolutions. *Tetrahedron Asymmetry* **1996**, *7*, 2633–2638. [CrossRef]