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ORIGINAL PAPER

Chemo-Enzymatic Synthesis of Enantiopure β-Blocker (S)-Metoprolol and Derivatives

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Abstract
(*S*)-Metoprolol, ((2 *S*)-1-[4-(2-methoxyethyl)phenoxy]-3-(propan-2-ylamino)propan-2-ol has been synthesised in 99% *ee* with high yield by a four step chemoenzymatic protocol. Several preparations of *Candida antarctica* lipase B have been screened in a kinetic resolution of the secondary chlorohydrin 1-chloro-3-(4-(2-methoxyethyl)phenoxy)propan-2-ol. We here report specific rotation values of the enantiopure chlorohydrins from the enzyme catalysed kinetic resolution, in addition to a specific rotation value for (*S*)-metoprolol, which determines the absolute configuration of the drug, and also the absolute configuration of the chlorohydrin enantiomers.

Keywords (S)-Metoprolol · Enantiopure building blocks · *Candida antarctica* Lipase B · Chiral chromatography · Specific rotation

1 Introduction

Cardiovascular disease is the top cause of death globally, and hypertension, or high blood pressure, and is estimated to be the cause of more than nine million deaths annually. In 2011, hypertension accounted for approximately 13% of all deaths worldwide [1]. Metoprolol is one of the most prescribed medications worldwide used in the treatment of hypertension and chest pain caused by restriction in blood supply to tissues, known as angina pectoris [2]. The drug was the 6th most prescribed drug in the US in 2020 with 66 million sold doses [3], and the metoprolol tartrate market size is estimated to reach nearly a billion USD by 2027 [4]. Metoprolol succinate is marketed as Toprol-XL® by AstraZeneca, and metoprolol tartrate as Lopressor® by Novartis. Both drugs are marketed with racemic API (Active Pharmaceutical Ingredient) [5]. Metoprolol tartrate was developed by Novartis and received approval in the United States August 7, 1978. Toprol-XL brand metoprolol succinate was developed by Astra Pharmaceuticals and the patent for Toprol was approved on January 14, 1992 [6]. Both preparations are used for treatment of hypertension and angina pectoris, however, metoprolol succinate can also be used in the treatment of heart failure. Side effects of metoprolol have been related to sleeping difficulties, feeling tired and abdominal discomfort of patients. Studies have shown that it is mainly the *R*-enantiomer of metoprolol that is responsible for these side effects [5, 7]. In addition, peripheral circulation disorder, blood glucose metabolism and altered intraocular pressure when using β-blockers have been reported to be caused by the *R*-enantiomers [8]. 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 [9]. The development of economical and sustainable methods for the production and analysis of enantiopure drugs have since then led to a high level of interest, and in many cases a "chiral switch" from racemic to enantiopure drugs [10]. Emcure (India) manufactures the pure *S*-enantiomer of metoprolol as METPURE-XL. The following advantages are listed in their advert: *[The drug] provides the beta-1 blocker component only, at half the racemate dose, avoiding the beta-2 blocking component, can be administered at high doses, safer in poor metabolizers of CYP2D6, avoids many drug-drug interactions.* This is also stated by Aneja et al. (2007) [11]. The sales numbers of the enantiopure drug

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is not known, but the advantages for patients are clear, and it is important to develop efficient and low cost synthesis protocols of (*S*)-metoprolol (Fig. 1).

Several other protocols to (*S*)-metoprolol are also patented [12, 13]. When other green chemistry principles are taken into consideration in addition to the use of a biocatalyst, such a protocol may be of industrial interest. In addition, Zhang et al. (2009) [14] have synthesized (*S*)-metoprolol in *ee* > 99% using a C12 higher carbon sugar as a chiral auxiliary. Hydrolytic kinetic resolution by use of Jacobsen's catalyst has also been used to produce (*S*)-metoprolol (in 96% *ee*) [15]. Borowiecki et al. (2022) have recently published a chemo-enzymatic approach to obtain (*S*)-metoprolol in high enantiomeric purity, however, the specific rotation of the product is not reported [16]. Soni et al. (2017) reported on a similar approach with several lipases as catalysts in the kinetic resolution to obtain the chiral building block 1-chloro-3-(4-(2-methoxyethyl)phenoxy)propan-2-ol for (*S*)-metoprolol, however, the authors claim that the remaining alcohol from the kinetic resolution of a racemate is the *S*-enantiomer (and not the *R*-enantiomer [17], as the Kazlauskas´s rule states [18], which we also have observed) and they report no optical rotation values for the products. Pandya et al. (2021) have published an article for chromatographic separation of metoprolol enantiomers on different chiral stationary phases and have given specific rotation values for the *S*-enantiomer of the drug, however not for the chiral building blocks [19]. We have previously published efficient protocols for several β-adrenergic blocking agents with focus on short reaction time and reduced use of reagents [20–24] compared to earlier literature, and we here present the lipase catalyzed synthesis of (*S*)-metoprolol. Even if the *E*-value of the kinetic resolution of these alcohol building blocks is high and a high *ee* of the wanted enantiomer is obtained, there will always be a 50% "waste" due to the unchanged alcohol of the starting material. This may be overcome with the use of dynamic kinetic resolution (DKR) [25]. However, we have not performed DKR in the present project. Assignments of absolute configuration of the alcohol enantiomers from lipase catalysed kinetic resolutions are important to determine, and we have previously determined absolute configuration on similar chiral compounds as the building blocks for metoprolol [26, 27].

2 Experimental Section

2.1 Chemicals and Reagents

All chemicals are commercially available and of analytic grade. The chemicals were bought from Sigma-Aldrich Norway, (Oslo, Norway). HPLC grade solvents were used for HPLC analyses. Dry solvents (tetrahydrofuran and acetonitrile) were prepared with a solvent purifier, MBraun MDSPS800. (München, Germany). Hexane was dried manually by adding molecular sieves (4Å) to the solvent 24 h before use.

2.2 Activation of Molecular Sieve

Molecular sieves (1/8 pellets, pore diameter 4Å) were placed in a porcelain dish and dried at 1000 °C for 24 h and kept in a desiccator thereafter.

2.3 Enzymes

Candida antarctica lipase B (CALB) (Novozym 435, 1–2% water content; activity ca. 10,000.

PLU/g, the ester synthesis activity of Novozym 435 is expressed in Propyl Laurate Units per gram (PLU/g). lot number LC200204) immobilized on a macroporous acrylic resin was gifted from Novozymes AS (Bagsværd, Denmark). *Candida antarctica* lipase B (activity ca. 4,000 PLU/g, (propyl laurate units/g, lot number BCBP3525V,).) immobilized on Immobead 150, recombinant from *Aspergillus oryzae* was purchased from Sigma-Aldrich Norway (Oslo, Norway). *Candida antarctica* Lipase B (CALB) (activity ≥ 10,000 PLU/g, 1 unit corresponds to the synthesis of 1 µmol per minute propyl laureate from lauric acid and 1-propanol at 60°C, lot#20,170,315), immobilized at high hydrophobic macroporous resin, produced in fermentation with genetically modified *Pichia pastoris* was gifted from SyncoZymes Co, Ltd. (Shanghai, China).

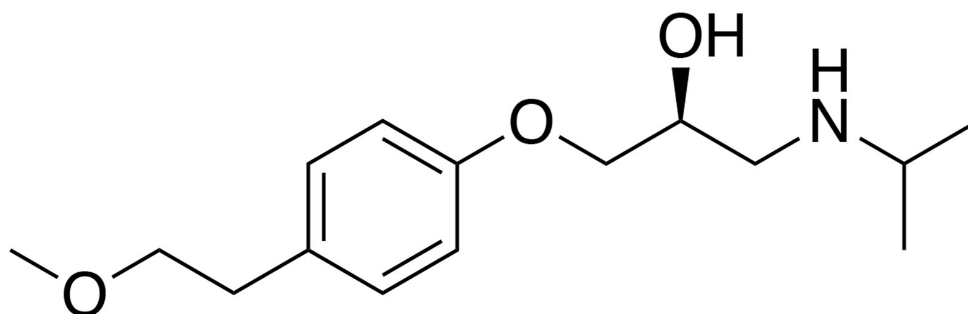


Fig. 1 (*S*)-metoprolol

2.4 General Analyses

TLC was performed on Merck silica 60 F_{254} and detected by UV at λ = 254 nm. Flash chromatography was performed on silica gel from Sigma-Aldrich (Oslo, Norway). Pore size 60 Å, 230–400 mesh particle size, 40–63 µm particle size. NMR analyses were recorded on a Bruker 600 MHz Avance III HD system equipped with a 5-mm cryogenic CP-TCI z-gradient probe from Bruker BioSpin GmbH (Karlsruhe, Germany). Mass spectroscopy (MS): Accurate mass determination in positive and negative mode was performed on a "Synapt G2-S" Q-TOF instrument from Waters™ (Waters Norway, Oslo, Norway). Samples were ionized by the use of ASAP probe (APCI). Calculated exact mass and spectra processing was done by Waters™ Software (Masslynxs V4.1 SCN871). Infrared spectroscopy was performed on a NEXUS FT-IR model 470 instrument from Thermo Nicolet Corporation (Madison, WI, USA). Optical rotation was determined on a PerkinElmer Model 341 Polarimeter (Waltham, MA, USA), with a cell of 10 cm length, λ 589 nm. The enzymatic reactions were performed in a New Brunswick G24 Environmental Incubator Shaker from New Brunswick Co. Inc. (Edison, NJ, USA) or in an Infors Minitron (Infors AG, Bottmingen, Switzerland).

2.5 Chiral HPLC Analyses

HPLC analyses of **1a** were performed on an Agilent HPLC 1100 with a manual injector (Rheodyne 77245i/Agilent 10 µl loop). A Chiralcel OD-H column from Daicel, Chiral Technologies Europe (Gonthier d´Andernach, Illkirch, France) was used (250 mm, i.d. 4.6 mm). Method was hexane:2-propanol 83:17, 0.6 mL min^{-1}, UV 254 nm. t_R (*S*)-**1a** = 14.354 min, t_R (*R*)-**1a** 16.714 min.

2.6 Chiral Gas Chromatography Analyses

GLC-analyses of **2** were performed on a Varian 3380 instrument with an autosampler. A split injector (200 °C) was used with a flame ionization detector (FID, 250 °C). The column used was a CP Chirasil DEX column from J&W Scientific (Santa Clara, CA, USA) (25 m, i.d. 0.25 mm, d_f 0.25 µm). Method: gas pressure 8 psi, split flow 60 mL min^{-1}, temp. prog. 100–130 °C (10 °C/min), 130–150 °C (1 °C/min), 150–190 °C (10 °C/min, hold 10 min), 190–200 °C (0.5 °C/min). t_R = 47.172 min (*R*)-**2**); t_R=47.469 min ((*S*)-**2**).

2.7 Assignment of Absolute Configurations

Absolute configuration of the faster reacting enantiomer in lipase catalyzed resolution was determined by the known enantioselectivity of CALB [26] and by comparing the elution orders of the enantiomers with GLC elution orders of similar enantiopure compounds synthesised from (*S*)-epichlorohydrin [27]. It was observed from the chiral HPLC analyses of the kinetic resolution of **1a** that the enantiomers eluted in the opposite order compared to the chiral GLC analyses of enantiomers of **2**.

2.8 Syntheses of Racemic Compounds

2.8.1 1-Chloro-3-(4-(2-Methoxyethyl)Phenoxy)Propan-2-ol (1a) and Epoxide 1b [28]

4-(2-Methoxyethyl)phenol (10.0 g, 0.0657 mol) was placed in a 100 mL round bottom flask with MeOH (20 mL). To this solution KOH (85%, 4.50 g, 0.07 mol) was added and the mixture was stirred at 40–45 °C for 1 h. Approx. 7 mL of MeOH was distilled off under reduced pressure and epichlorohydrin (10 mL, 0.13 mol) was added. The reaction mixture was stirred at 40 °C for 48 h while being monitored by TLC (pentane:CH_2Cl_2:MeOH 10:9:1, R_f**1b** = 0.90). The mixture was washed with dist. H_2O (3 × 5 mL), dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to remove excess epichlorohydrin. This yielded 9.37 g of a mixture of **1a** (18%) and **1b** (82%) as a yellow oil. The amount of each compound was determined by GLC analysis.

2.8.2 1-Chloro-3-(4-(2-Methoxyethyl)Phenoxy)Propan-2-ol (1a)

To the 9.37 g mixture of **1a** and **1b**, LiCl (3.26 g, 0.077 mol), AcOH (6.60 mL, 0.12 mol) and dry THF (80 mL) were added. The reaction mixture was stirred for 48 h until TLC showed full conversion (pentane: CH_2Cl_2:MeOH 10:9:1, R_f **1a** = 0.65). Et_2O (50 mL) and H_2O (100 mL) were added, and the organic phase was washed with satd. $NaHCO_3$ and dried over anhydrous $MgSO_4$. The mixture was purified by column chromatography (eluent: pentane: CH_2Cl_2:MeOH 10:9:1). This yielded the racemic alcohol **1a** (6.90 g, 0.028 mol, 43% yield, 100% purity (NMR) as a colorless oil. ^{1}H NMR ($CDCl_3$, 600 MHz, *δ*): 7.15 (d, 2 H, aromatic); 6.85 (d, 2 H, aromatic); 5.30 (s, -OH); 4.20 (sx, 1 H, -CH-); 4.06 (m, 2 H, OCH2-); 3.78 and 3.70 (dd, 1 H, -CH_2Cl); 3.57 (t, 2 H, OCH-); 3.35 (s, 3 H, CH_3-); 2.82 (t, 2 H, -CH_2-). ^{13}C NMR: 156.7; 132.0; 129.9; 114.5; 73.8; 69.9; 68.6; 58.7; 46.0; 35.3 [14, 19]. (The structure was also confirmed by COSY, HSQC and HMBC, and IR (cm^{-1}, pure): 3403 (br, OH stretch), 2929 − 2869 (m, br, C-H stretch), 1510 (s, aromatic C = C stretch), 1240 (s, C-O stretch), 1109 (s, C-O stretch), 826 (s, C-Cl stretch) MS (TOF-ASAP): M^+ 244.0862 *m/z*. (Calcd. 244.7133) HPLC: t_R (*S*)-**1a** = 14.35 min, t_R (*R*)-**1a** 16.71 min. (See supporting information for all spectra of **1a**).

2.8.3 Characterisation of (2-((4-(2-Methoxyethyl)Phenoxy)Methyl)Oxirane) **1b**

After purification of the reaction mixture of **1a** and **1b** by column chromatography (pentane: CH_2Cl_2:MeOH 10:9:1), the epoxide **1b** was purified (99% purity, NMR) and characterized. ^{1}H NMR ($CDCl_3$, 600 MHz, *δ*): 7.13 (d, 2 H, aromatic); 6.85 (d, 2 H, aromatic); 3.93 and 4.18 (dd, 2 H, -$PhOCH_2$-); 3.56 (t, 2 H, -OCH_2-); 3.34 (s, 3 H, CH_3-); 3.33 (m, 1 H, -CH-); 2.82 (t, 2 H, -CH_2-); 2.74 and 2.90 (dd, 2 H, -$CHCH_2O$-). ^{13}C NMR: 157.0; 131.7; 129.8; 114.6; 73.8; 68.8; 58.6; 50.2; 44.7; 35.3. The structure was also confirmed by COSY, HSQC and HMBC, and IR (cm^{-1}, pure): 2924 − 2869 (m, br, C-H stretch), 1510 (s, aromatic C = C stretch), 1239 (s, C-O stretch), 1109 (s, C-O stretch), 828 (s, ring deformation). MS (TOF-ASAP): M^+ 208.1096 *m/z*. (See supporting information for all spectra of **1b**).

2.8.4 Synthesis of 1-Chloro-3-(4-(2-Methoxyethyl)Phenoxy)Propan-2-yl Butanoate, **2** (Derivatization)

A small vial was charged with 1-chloro-3-(4-(2-methoxyethyl)phenoxy)propan-2-ol (**1a**) (18.0 mg, 0.06 mmol), butanoic acid anhydride (2 drops), pyridine (5 drops) and n-hexane (0.5 mL). The vial was placed in an air bath at 60ºC for 1 h. The solution was washed with dist. H_2O (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 on GLC, with CP Chirasil DEX column, gas pressure 8 psi, split flow 60 mL min^{-1} and temp. prog. 100–130 °C (10 °C/min), 130–150 °C (1 °C/min), 150–190 °C (10 °C/min, hold 10 min), 190–200 °C (0.5 °C/min). Enantiomers of **2**: t_R = 47.172 min (*R*); t_R = 47.469 min (*S*). R_s = 1.55.

2.9 Enzymatic Kinetic Resolution of Racemates

2.9.1 General Procedure

Racemic substrate (4.36×10^{-5} mol) and vinyl butanoate (2.19×10^{-4} mol) were added to a closed vial with dry hexane (3 mL). The reaction was started by adding enzyme (20 mg) and the vial was placed in an incubator shaker (30 °C, 200 rpm). Samples of 150 µL were extracted at periodic intervals and analyzed by HPLC and GLC. The *E*-values and K_{eq} were calculated by the software program *E&K Calculator 2.1b0 PPC* [29].

2.9.2 Small Scale Transesterification of **1a** with CALB

In dry hexane (3 mL), **1a** (0.0106 g, 4.36×10^{-5} mol) and vinyl butanoate (0.025 g, 2.19×10^{-4} mol) were added as described in Sect. 2.9.1. CALB (25 mg) was then added and the reaction vial was placed in an incubator shaker (30 °C, 200 rpm). Samples were extracted every 30 min for the first two hours, thereafter every hour until 50% conversion (5–48 h). Novozym 435, CALB from Sigma-Aldrich and the CALB preparation from Syncozymes were used, and the reactions were all run in parallel.

2.9.3 Large Scale Enzymatic Kinetic Resolution of **1a** with CALB

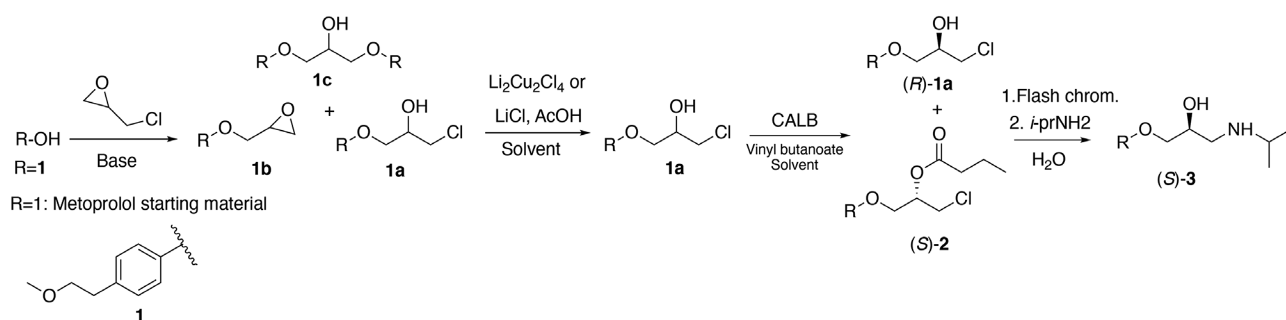
Alcohol **1a** (1.35 g, 5.53 mmol) and vinyl butanoate (3.34 g, 0.29 mol) were added to a flask with dry hexane (130 mL) and molecular sieves (4Å). CALB (Syncozymes) (1.78 g) was added and the reaction mixture was incubated (30 °C, 200 rpm) for 8 h before the enzyme and molecular sieves were filtered off and the solvent was evaporated under reduced pressure. The ester (*S*)-**2** and alcohol (*R*)-**1a** were separated by flash chromatography (pentane: CH_2Cl_2:MeOH, 10:9:1). This yielded (*S*)-**2** (0.889 g, 2.76 mmol, 50% yield). GLC t_R (*R*)-**2** = 47.172 min and t_R (*S*)-**2** = 47.469 min and (*R*)-**1a** (0.409 g, 1.67 mmol, 30.5% yield, 99% purity). HPLC t_R (*R*)-**1a** = 16.056 min, *ee* = 99% $[\alpha]_D^{20} = -4.0$. (c 1.0, MeOH).

2.9.4 Synthesis of (*S*)-**1a** by CALB Catalyzed Hydrolysis of (*S*)-**2**

The ester (*S*)-**2** (0.455 g, 1.45 mmol) was transferred to a vial with sodium phosphate buffer (6 mL, pH 7.0) and molecular sieves. Novozym 435 (1.13 g) was added and the reaction mixture was incubated in an incubator shaker (30 °C, 200 rpm) for 24 h. The enzyme and molecular sieves were filtered off and the solvent was removed under reduced pressure. The alcohol (*S*)-**1a** was isolated by flash chromatography (pentane: CH_2Cl_2:MeOH, 10:9:1). This yielded (*S*)-**1a** (0.287 g, 1.24 mmol, 86% yield). HPLC t_R = 14.166 min, *ee* = 99.9%. $[\alpha]_D^{20} = +4.0$. (1.0, MeOH).

2.9.5 Synthesis of (*S*)-Metoprolol ((*S*)-**3**)

To (*R*)-**1a** (99% *ee*) (0.409 g, 1.62 mmol), isopropylamine (5 mL) and dist. H_2O (1.5 mL) was added. The reaction was stirred at room temp. for 48 h until TLC (pentane:CH_2Cl_2:MeOH, 10:9:1, Rf, (*S*)-**3** = 0.45) showed full conversion. This gave (*S*)-**3** (0.278 g, 1.04 mmol, 62% yield, 95% purity, NMR) as a colorless oil. HPLC: t_R = 19.89 min, *ee* = 99% $[\alpha]_D^{20} = -39.78$(c 0.88, MeOH). ^{1}H



Scheme 1 Synthesis of (*S*)-metoprolol ((*S*)-**3**) *via* a four step route including CALB catalysed kinetic resolution of 1-chloro-3-(4-(2-methoxyethyl) phenoxy)propan-2-ol, **1a**

NMR ($CDCl_3$, 600 MHz, δ): 7.13 (d, 2 H, aromatic); 6.83 (d, 2 H, aromatic); 5.30 (s, NH); 5.22 (s, OH); 4.33 (m, 1 H, CHOH); 4.06–3.95 (m, $PhOCH_2$); 3.56 (t, 2 H, $-OCH_2-$); 3.35 (s, 3 H, CH3); 3.17 (sp, 1 H, -CH-); 3.09–2.96 (m, 2 H, $-CH_2NH-$); 2.82 (t, 2 H, $-CH_2Ph-$); 1.29 (d, 3 H, CH_3); 1.29 (d, 3 H, CH_3). ^{13}C NMR: 156.9; 131.7; 129.8; 114.5; 73.8; 70.6; 66:2; 58.7; 50.1; 48.5; 35.3; 19.3. IR (cm^{-1}, pure): 3387 (br, OH stretch), > 3000 (br, secondary amine N-H stretch), 2960 – 2872 (m, br, C-H stretch), 1548 (s, aromatic C = C stretch), 1396 (s, C-O stretch), 1243 (s, C-O stretch), 1096 (s, C-N stretch). HRMS (APCI-ASAP): $[M + H]^+$ 268.191 *m/z*. (Calcd. 267.364 $C_{15}H_{25}O_3N$) [14, 19].

3 Results and Discussion

3.1 Synthesis of Racemic Building Blocks

The racemic building block 1-chloro-3-(4-(2-methoxyethyl) phenoxy)propan-2-ol (**1a**) was synthesised according to Scheme 1 by deprotonation of 4-(2-methoxyethyl)phenol by 85% potassium hydroxide in methanol according to Regla et al. (2008) [28]. The corresponding epoxide **1b** was also formed, which may proceed *via* two different mechanisms, which we have predicted in Gundersen et al. [21]. By using stoichiometric amount of the base, formation of the dimer **1c** was avoided, compared to when excess of the base was used. Long reaction times and high temperatures will also favor formation of the dimer by-product. Shorter reaction time will also avoid the intramolecular formation of the epoxide **1b**.

After 48 h reaction time, GLC analysis showed a 18/82 ratio of the formed chlorohydrin **1a** to the corresponding epoxide **1b**. The ring opening of **1b** was first performed as described by Lund et al. [20] for a reaction with similar reactants and products, with a yield of 29%. However, when lithium chloride and acetic acid were used (see experimental part), a yield of **1a** of 43% was obtained.

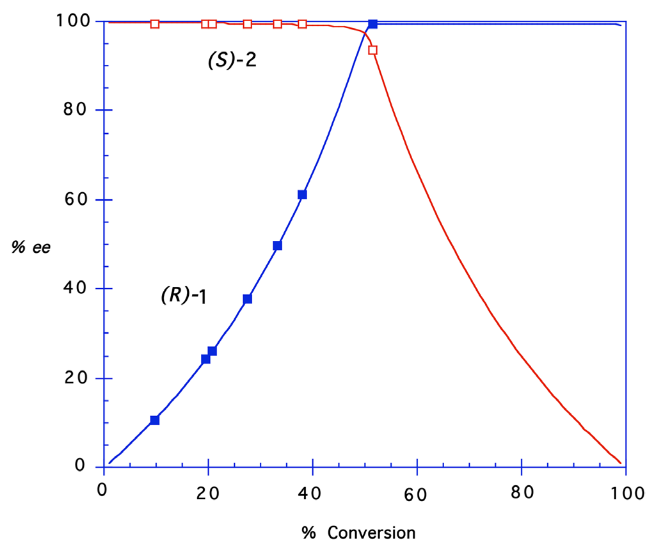


Fig. 2 Reaction progress of the kinetic resolution (transesterification) of **1a** with Novozym 435 in dry hexane with vinyl butanoate as acyl donor, *E* = > 200. Reaction time to 50% conversion was 48 h. The blue curve correlates to the enantiomeric excess of the remaining substrate (ee_s, (*R*)-**1**), while the red curve correlates to the enantiomeric excess of the product (ee_p, (*S*)-**2**). The open and closed squares represent the experimental values, the lines are generated values from the computer program *E&K Calculator 2.1.b0 PPC* [29]. A similar graph was obtained when the esterification was performed with CALB from Syncozymes, however, this reaction reached 50% conversion after 8 h, and this enzyme preparation was chosen for the largescale kinetic resolution of **1a**

3.2 Kinetic Resolution of 1a Catalysed by Lipase B from *Candida Antarctica*

Three enzyme preparations of CALB have been used to resolve 1-chloro-3-(4-(2-methoxyethyl)phenoxy)propan-2-ol (**1a**): Novozym 435 from Novozymes AS (Bagsvaerd, Denmark), one CALB preparation purchased from Sigma-Aldrich (Oslo, Norway) and CALB from Syncozymes LTD (Shanghai, China). All three preparations gave 99% enantiomeric excess (*ee*) of the wanted chiral building block (*2R*)-1-chloro-3-(4-(2-methoxyethyl)phenoxy)propan-2-ol, (*R*)-**1a**), (see Fig. 2). However, CALB from Syncozymes

entailed a faster reaction time (8 h) compared to the preparations from Novozymes and Sigma-Aldrich (both 48 h). The enantiomers of alcohol **1a** were resolved by HPLC on Chiralcel OD-H column, with eluent hexane:2-propanol, 83:17, flow: 0.6 mL min^{-1}, UV 254 nm, t_R = 14.354 min (*S*)-**1a**); 16.714 min (*R*)-**1a**). (Fig. 15 in S. I.). (*R*)-**1a** was obtained in 61% yield and 99% purity in 99% *ee* and the specific rotation was $[\alpha]_D^{20} = -4.0$. (c 1.0, MeOH). The ester **2** was resolved by GLC on a Chirasil Dex column with gas pressure 8 psi, split flow 60 mL min^{-1}, temp. prog. 100–130 °C (10 °C/min), 130–150 °C (1 °C/min), 150–190 °C (10 °C/min, hold 10 min), 190–200 °C (0.5 °C/min). t_R = 47.172 min (*R*); t_R = 47.469 min (*S*). R_s = 1.55.

3.3 Hydrolysis of (*S*)-2 Catalysed by Lipase B from *Candida Antarctica*

The ester (*S*)-**2** produced in the transesterification reaction was hydrolysed by Novozym 435 in phosphate buffer and gave the alcohol (*S*)-**1a** in 99.9% *ee*, Scheme 2.

The specific rotation of (*S*)-**1a** was $[\alpha]_D^{20} = +4.0$. (c 1.0, MeOH), which is in accordance with the mirror image (*R*)-**1a**. The absolute configuration of (*R*)-**1a** and (*S*)-**1a** is based on these specific rotation values and also on the preference of CALB for secondary alcohols with one large group and one small group connected to the stereocenter [26, 27]. Since the enantiomers of the butanoate ester **2** were not possible to separate on HPLC, this led to time consuming analyses since each sample had to be analysed on both HPLC and GLC. For the separation of the racemic ester many different temperature programs, gas pressures and split flow values were tested in order to achieve a R_s-value of 1.55. A low concentration of the sample was necessary to achieve good separation. The method used for separation of **2** was gas pressure 8 psi, split flow 60 mL min^{-1} and temp. prog. 100–130 °C (10 °C/min), 130–150 °C (1 °C/min), 150–190 °C (10 °C/min, hold 10 min), 190–200 °C (0.5 °C/min). The racemic ester **2** was prepared by derivatisation of racemic **1a** with butanoic anhydride and the separation of **2** is shown in Supplementary Information as Fig. 16.

The lipase preparation from Syncozymes was chosen for the largescale kinetic resolution due to its shorter reaction time. The reaction time of a biocatalysed reaction is of course dependent of how much enzyme is used. The activity of an enzyme preparation is also known to be decreasing by time, even if the enzyme is stored at the correct temperature. We have previously re-used batches of Novozym 435 up to six times, the selectivity has shown to be preserved and the reaction time even increased from entry 1 to entry 6 [30].

3.4 Decreasing *E*-Value by Increasing Conversion of Transesterification Reaction of 1a

By calculating the *E*-value manually by calculator from the kinetic resolution of **1a** by inserting the measured ee_p and ee_s values into Eq. (1) [31],

$$E = \frac{\ln \frac{[ee_p(1-ee_s)]}{(ee_p+ee_s)}}{\ln \frac{[ee_p(1+ee_s)]}{(ee_p+ee_s)}} \tag{1}$$

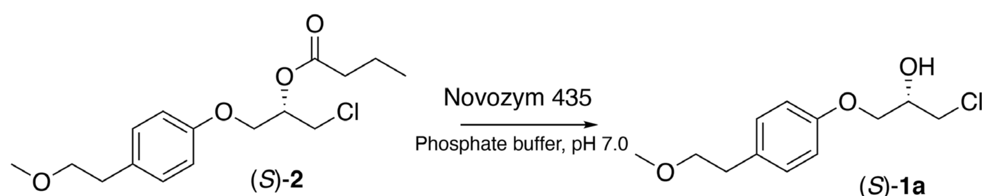
it was observed that the *E*-value decreased by increasing conversion (Fig. 3).

We have previously observed the trend of decreasing *E*-values in transesterifications with similar compounds (while the *E*-value increased in hydrolysis reactions of the corresponding butanoic esters) and that the selectivity of the enzyme increased when additional enantiomerically pure *R*-alcohols (the same as the substrate and similar *R*-alcohols) were added [32]. The explanation we came up with was that CALB may have an allosteric site that when these *R*-alcohols bind (reversibly) to this site, the active site alters. However, an allosteric site in CALB has not been reported, so we anticipate that this binding is formed with one of the amino acids on the surface of the enzyme.

3.5 Synthesis of (*S*)-Metoprolol, (*S*)-3

Enantiopure (*S*)-metoprolol ((*S*)-**3**) was obtained *via* the reaction of (*R*)-**1a** (99% *ee*) with isopropylamine in methanol according to Scheme 1. The *ee* of the *R*-chlorohydrin was retained in the product, Fig. 4; Table 1. With eluent hexane:2-propanol 83:17, flow: 0.6 mL min-1, UV 254 nm. t_R= 19.889 ((*S*)-**3**).

(*S*)-Metoprolol ((*S*)-**3**) was obtained with 95% purity, 99% *ee* in 62% yield. The specific rotation value of (*S*)-**3** was $[\alpha]_D^{20} = -39.7$ (c 0.88, MeOH), whereas Pandya et al. (2021) [19], have measured it to $[\alpha]_D^{25} = -30.2$ (c 1.0, EtOH) for 99.4% *ee*. The specific rotation value determined by Pandya et al. and our specific rotation value are measured under slightly different temperatures and different concentration



Scheme 2 Hydrolysis of (*S*)-**2** by Novozym 435 in phosphate buffer pH 7.0 yielding (*S*)-**1a** in 99.9% *ee*

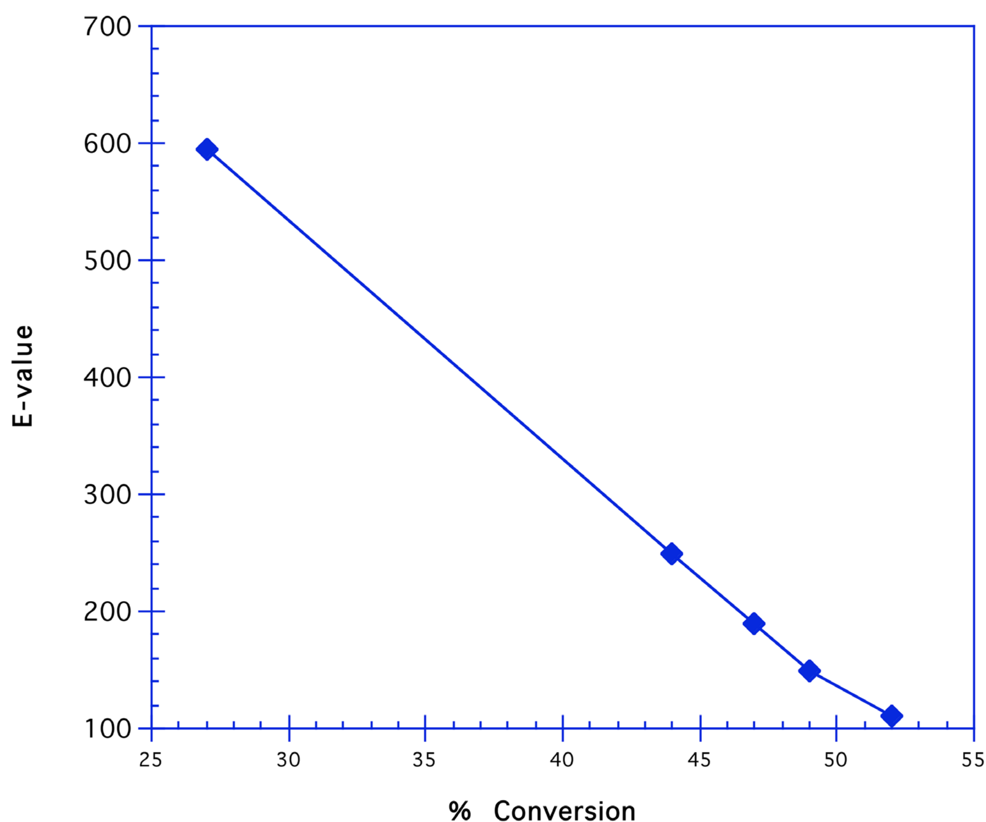


Fig. 3 Decreasing E-value from $E=590$ to $E=110$ with increasing conversion from 27–52% in the transesterification reaction of **1a** with Novozym 435, vinyl butanoate as the acyl donor in dry hexane. The average E-value was calculated to 200 by *E&K Calculator 2.1.b0 PPC* [29]. The same trend was observed with CALB from both Sigma-Aldrich and from Syncozymes

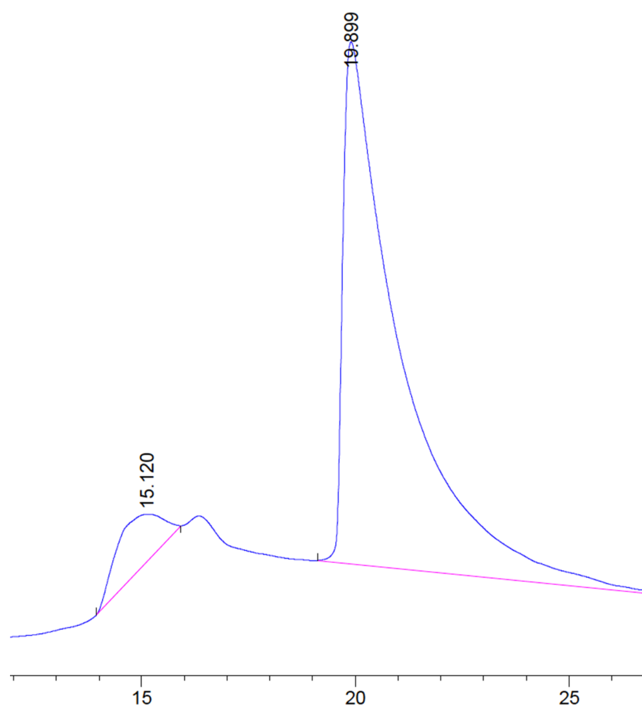


Fig. 4 Chromatogram of (*S*)-metoprolol, (*S*)-**3**, 99% *ee*. The column was a Chiralcel OD-H column with eluent hexane:2-propanol 83:17, flow: 0.6 mL min-1, UV 254 nm. t_R= 19.889 ((*S*)-3).

and solvents, therefor we mean that both values are correct. Mutukrishnan et al. (2007) report specific rotation value of (*S*)-metoprolol in 96% *ee* as a colorless solid of $[\alpha]_D^{25}=-8.10$ (c 10.0, $CHCl_3$) [15]. As we know, specific rotation values can only be compared when the concentration, the *ee*, the temperature and the solvent are similar. The absolute configuration of our enantiopure (*S*)-**3** is based on these specific rotation values, and also on the specific rotation values of (*R*)-**1a** and (*S*)-**1a** based on the preference of CALB as mentioned above.

The ^{1}H NMR and ^{13}C NMR spectra of (*S*)-**3** which we present in the supplementary information show several impurities. The sample was purified before the optical rotation analysis, however, due to small amount of the product we were not able to achieve satisfying NMR spectra. The large proton signals at 0.93 and 3.66 ppm, as well as the carbon signals at 43.4 and 22.3 ppm, are assigned to isopropylamine. Traces of ethyl acetate can be seen as signals at 1.18 and 2.10 ppm in the 1 H NMR spectrum as well as signals at 61.6 and 14.0 ppm in the ^{13}C NMR spectrum. Hexane appears as a large proton signal at 1.28 ppm almost overlapping with the doublet from the protons in positions 14 and 15 and as carbon signals at 22.9 and 14.0 ppm. Acetone residues from the washing of the NMR tube is seen at 2.20 ppm. The rest of the non-integrated signals are from **1a** and epichlorohydrin, which have similar shifts and overlap with the shifts of (*S*)-**3**. Chemical shifts matching these compounds

Table 1 *E*-values and specific rotation values for pure enantiomers from kinetic resolutions of the secondary alcohol **1a** (X = Cl) with CALB from Syncozymes and vinyl butanoate as acyl donor. (*R*)-**1a** was treated with isopropylamine to give the final drug (*S*)-metoprolol ((*S*)-**3**) in 99% *ee* (X = isopropylamine). For further details, see experimental section

Substrate, X.	*E*-value	Alcohol, % *ee*	$[\alpha]_D^{20}$	Rx time to 50% conv. (h)
1a, Cl	> 200	(*R*)-**1a**, 99 (*S*)-**1a**, 99	–4.0, (MeOH) + 4.0 (MeOH)	8
(*R*)-1a, *i*-prNH		(*S*)-**3**, 99	–39.26, (EtOH)	-

are also found in the ^{13}C NMR spectrum. The large signal at 1.6 ppm originates from water. Otherwise, all spectra are in accordance with references 14 and 19.

The ion M + H was observed for (*S*)-**3** by MS (see Fig. 25 in supplementary information). The experiment was performed on a Synapt G2-S Q-TOF instrument fromWaters™. The sample was ionized by use of the ASAP probe (APCI). The exact mass of (*S*)-**3** was calculated to 267.18 g/mol by Waters™ Software (Masslynxs V4.1 SCN871), this fits well with the observed ion of 268.1911 g/mol, since the ion observed was M + H. The hydrogen deficiency index (HDI) is 3.5. The expected HDI is 4 as metoprolol contains one ring and three double bonds, however since the ion observed is M + H the DBE (double bond equivalents) becomes 3.5 as stated in the spectrum. The IR spectrum of (*S*)-**3** (Fig. 24 in supplementary information), is also in accordance with precious reported data [14, 19].

4 Conclusion

The enantiopure chlorohydrin (*R*)-**1a** was produced in 99% *ee* and 30.5% yield *via* a CALB catalyzed kinetic resolution of **1a**. Specific rotation values for (*R*)-**1a** and (*S*)-**1a** have not been reported previously. The amination reaction of (*R*)-**1a** gave (*S*)-metoprolol ((*S*)-**3**) in 62% yield and 99% *ee*. The absolute configuration of the final drug was determined by comparing the specific rotation values with previously reported data. The overall yield from the starting phenol **1** to (*S*)-**3** was approximately 20%. This protocol for (*S*)-metoprolol is produced in high enantiomeric purity however the total yield can be improved.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11244-023-01860-1.

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Declarations

Competing Interest The authors declare that they have no competing financial interests or personal relationships that could influence the work reported in this paper.

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