Article

Lipase Catalysed Synthesis of Enantiopure precursors and derivatives for β-Blockers Practolol, Pindolol and Carteolol.

Morten A. Gundersen [a], Guro Buaas Austli [a], Sigrid Sløgedal Løvland, Mari Bergan Hansen, Mari Rødseth [a] and Elisabeth Egholm Jacobsen [a, *]

[a] Department of chemistry, Norwegian University of Science and Technology, Høgskoleringen 5, 7491 Trondheim

* elisabeth.e.jacobsen@ntnu.no

Abstract: The β-blocker (*S*)-practolol ((*S*)-*N*-(4-(2-hydroxy(isopropylamino)propoxy)phenyl)acetamide) was synthesized with 96% enantiomeric excess (*ee*) from the chlorohydrin (*R*)-N-(4-(3-chloro-2 hydroxypropoxy)phenyl)acetamide, which was produced in 97% ee and with 27% yield. Racemic building block 1-((1H-indol-4-yl)oxy)-3-chloropropan-2-ol for the β-blocker pindolol was produced in 53% yield and (*R*)-1-((1H-indol-4-yl)oxy)-3-chloropropan-2-ol was produced in 92% *ee*. The chlorohydrin 7-(3-chloro-2-hydroxypropoxy)-3,4-dihydroquinolin-2(1*H*)-one, a building block for a derivative of carteolol was produced in 77% yield. (*R*)-7-(3-chloro-2-hydroxypropoxy)-3,4-dihydroquinolin-2(1*H*)-one was obtained in 96% *ee*. The *S*-enantiomer of this carteolol derivative was produced in 97% *ee* in 87% yield. Racemic building block 5-(3-chloro-2-hydroxypropoxy)- 3,4-dihydroquinolin-2(1H)-one, building block for the drug carteolol was also produced in 53% yield, with 99% *ee* of the *R*-chlorohydrin (*R*)-5-(3-chloro-2-hydroxypropoxy)-3,4-dihydroquinolin-2(1*H*)-one. The yield of all four chlorohydrins increased by use of catalytic amounts of base. The reason for this was found to be less formation of the dimeric by-products compared to use of higher concentration of the base. An overall reduction of reagents and reaction time was also obtained compared to our previous reported data of similar compounds. The enantiomers of the chlorohydrin building blocks were obtained by kinetic resolution of the racemate in transesterification reactions catalyzed by *Candida antarctica* Lipase B (CALB) from SyncoZymes Co, Shanghai, China. Optical rotations confirmed the absolute configuration of the enantiopure drugs.

Keywords: (*S*)-Practolol; paracetamol; (*S*)-pindolol; (*S*)-carteolol; *Candida antarctica* Lipase B; chiral chromatography; dimer formation

1. Introduction

Chiral compounds with one or several stereogenic centra consist of pairs of enantiomers. Many drugs on the marked today have one or several stereogenic centra, β-blockers normally have one stereogenic center, and then consist of two enantiomers. The enantiomers may have the same effect on the patient, or the enantiomers may have different effects, or worse, one enantiomer may have several unwanted side effects. FDA considers the «wrong» enantiomer as an impurity and demands for pure enantiomers as the active pharmaceutical ingredient (API) in the marketed drugs, not racemates. The demand for enantiomerically pure drugs has increased by the years from the 1990´s when FDA demanded manufacturers to evaluate the pharmacokinetics of a single enantiomer or mixture of enantiomers in a chiral drug. Quantitative assays for individual enantiomers should be developed for studies in *in vivo* samples early in drug development.

High blood pressure (hypertension) and heart failure are big global health problems. In Norway approximately 30% of all deaths are caused by cardiovascular diseases [1]. Approximately 26 million people world-wide lives with heart failure, and approximately seven million deaths are caused by hypertension annually [2]. Heart failure cannot be

cured but may be treated by medications in order to increase quality of life for the patients. Both heritage (genetic) and lifestyle may lead to increased risk of cardiovascular diseases. Risk factors for cardiovascular diseases, such as less activity, eating more sugar and salt, increased stress, obesity and overweight may be reasons for the increasing health problem worldwide [3].

A class of drugs that have been used in treatment of cardiovascular diseases for a long time, β-adrenergic receptor antagonists, so-called β-blockers. The β1-receptors in the human body are mainly located in the heart and regulate the contraction of the heart muscle. The β1-blockers are antagonists that affect the β1-receptors in the heart and are suitable for the treatment of hypertension and heart failure. When a β-blocker inhibits the binding of adrenaline and noradrenaline, the stress hormone level in the body will decrease, and thus the blood pressure and heart rate will decrease. β2-Receptors are mainly found in the lungs, and most β2-agonists are mainly used to treat asthma by relaxing the smooth muscle in the lungs [4].

Some β-blockers are non-selective and will inhibit both β1- and β2-receptors, while others are selective to either β1- or β2-receptors. Propranolol is an example of a so called non-selective β-blocker. A problem for the non-selective β-blockers is that they may cause negative effects to the lungs, especially for asthma patients. β-Receptors are found in all parts of the nervous system, and β-blockers will therefore give side-effects even if the right drug is chosen. Selective β-blockers lead to fewer side-effects and are safer in use. Choosing the right β-blocker for every type of treatment is therefore important [4].

We have for some time worked with optimization of biocatalytic processes for several of these drugs. Lipase catalyzed kinetic resolution of racemates has been successful for several of these. [5, 6] In attempts to obtain enantiopure building blocks for the β-agonist (*R*)-clenbuterol asymmetrizations of ketones have been performed with ketoreductases giving high enantiomeric excess. [7]

The *S*-enantiomers of the β-blockers are in general known to be more active than the *R*-enantiomers, and opposite stereo configuration for the β-agonists. Common for these compounds is a side chain on an aromatic group which consist of a secondary alcohol and an amine on the omega carbon. Traditionally most of these drugs have been manufactured with the racemate as the API, however, f. inst. (*S*)-atenolol, (*S*)-propranolol and (*S*)-metoprolol are also on the market as single enantiomers. When referring to a drug, one should refer to the API [8].

Because enantiomers are mirror images of each other and not identical, each of them may act differently on the receptor. The pharmacological effect, clinical effect and toxicity of both enantiomers need to be investigated separately according to the U.S Food and Drug Administration (FDA) [2]. Chiral drugs are today promoted increasingly with enantiopure API. For enantiopure drugs it is a requirement of an enantiomeric excess (*ee*) > 96%. Therefore, development of environmentally friendly synthetic methods for each of the enantiomers is important.

Practolol is a selective β1-antagonist and was the first β1-selective β-blocker used in the treatment of cardiovascular diseases in the beginning of the 1970´s [9]. Practolol showed effective treatment of heart failure and arrhythmic heart rate [10]. The drug showed later some critical side-effects, such as culomucocutaneous syndrome, in some patients, and was withdrawn from the market.[11].

Mulik *et al.* reported in 2016 a four-step synthesis of (*S*)-practolol in 100% *ee* with use of *Pseudomonas cepacia* sol-gel AK lipase as enantioselective catalyst. However, they report that the produced ester from the transesterification reaction is hydrolyzed and aminated to give the enantiopure building block in *S*-configuration [12]. According to previous reports it is the slower reacting enantiomer (*R*)-*N*-(4-(3-chloro-2-hydroxypropoxy)phenyl)acetamide which is aminated to give (*S*)-practolol. They also claim that the stereo configuration of the chlorohydrin is inverted in the amination, which will not be the case since the amine is not attacking the stereocenter, but the primary carbon with the chloro atom. Ader and Schneider reported in 1992 enzymatic kinetic resolution of racemic *N*-(4-(3-

chloro-2-hydroxypropoxy)phenyl)acetamide with *Pseudomonas* sp. to give (*R*)-*N*-(4-(3-chloro-2-hydroxypropoxy)phenyl)acetamide which was subsequently aminated to yield (*S*)-practolol in 30% yield in >99% *ee* [13]. However, no optical rotation values of the building blocks or the drug have been reported. In order to develop greener and more sustainable processes for drugs with secondary alcohol side chains we wanted to include the synthesis of practolol despite the regulations of the drug.

1-(1H-indol-4-yloxy)-3-(isopropylamino)-2-propanol was first released for clinical use in USA in 1982. It is a non-selective β-blocker, used in the treatment of high blood pressure, chest pain and irregular heartbeat. Pindolol has its substituents on the aromatic ring in the ortho- and meta-position, which is common for non-selective β-blockers. Selective β-blockers usually have a substituent in the para-position on the aromatic ring [4]. Pindolol is also a partial agonist and will therefore slow the resting heart rate less than other β-blockers like atenolol or metoprolol [14]. Pindolol is usually sold under the brand names Visken (Sandoz) or Barbloc (Alpha) and is often used to treat high blood pressure during pregnancy because it does not affect the foetal heart function or blood flow. Although pindolol is a non-selective β-blocker, other uses for the drug have been reported. It has been tested in the treatment of fibromyalgia and related fatigue diseases, as well as in the treatment of depression in combination with selective serotonin reuptake inhibitors [15, 16] Pindolol is a rapidly absorbed drug, and after oral ingestion it can be detected in the blood after 30 minutes. In patients with normal renal function pindolol has a plasma half-life of three to four hours. The drug is also lipophilic and enters the central nervous system rapidly. Reported side effects include unwanted lowering of heart function or changes in the respiratory system. These side effects are related to its β-adrenergic blocking activity, other side effects have also been reported, such as dizziness, vivid dreams, feeling of weakness or fatigue, muscle cramps as well as nausea [14]. Precursors of β-blocker pindolol were reported synthesized by biocatalysis in 2017 by Lima *et al.* They perfomed hydrolysis of precursor 2-acetoxy-1-(1H-indol-4-yloxy)-3-chloropropane using lipase from *Pseudomonas fluorescens* which yielded (2*S*)-1-(1H-indol-4-yloxy)-3-chloro-2-propanol in 96% *ee* and (2*R*)-2-acetoxy-1-(1H-indol-4-yloxy)-3-chloropropane in 97% *ee,* which was hydrolysed giving 97% *ee* of the *R*-chlorohydrin for synthesis of (*S*)-pindolol with retention of *ee* [15]. However, we have some doubts about the stereochemistry in this report which will be discussed.

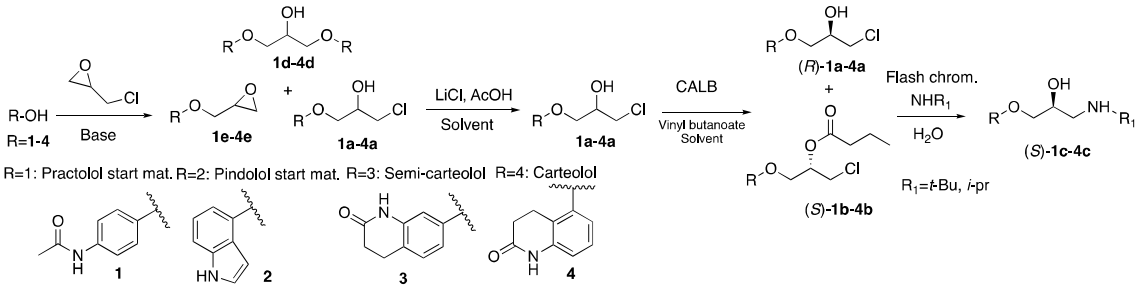
Carteolol is another β-adrenergic antagonists (β-blocker) manufactured mostly with racemic API and administered as eye-drops for reduction of aqueous production in the eye (glaucoma). [17]. In these patients an elevated intraocular pressure (IOP) leads to damage to the optic nerve, reducing the visual field gradually until the patient is completely blind. It is the second most common cause of irreversible blindness after age-related macular degeneration in western Europe. In 2010, 2.1 million people worldwide went irreversibly blind because of glaucoma [18]. In 2019, the majority of Norwegian glaucoma patients (68%) were treated with β-blockers betaxolol or timolol, either as single drugs or in combination with other drugs such as prostaglandin analogues or carboanhydrase inhibitors [19].

With the aim of sustainable production of enantiopure β-blockers we have performed several synthesis strategies with lower amounts of reactants and lower reaction times than previously reported. The general mechanism of base catalyzed deprotonation of phenolic protons with subsequent nucleophilic attach of epichlorohydrin has been studied with different bases and different concentrations of epichlorohydrin. Lipase B from *Candida antarctica* has shown to catalyse reactions of similar compounds with high *ee* of both product and remaining starting material (hydrolysis and transesterification reactions) [20, 21].

2. Results

Chlorohydrin building blocks (*R*)-**1a**-**4a** for synthesis of enantiomers of the β-blockers practolol ((*S*)-**1c**), pindolol and derivatives of carteolol ((*S*)-**3c-4c**), have been synthesized in 92-97% *ee* by chemo-enzymatic methods (Scheme 1, Table 3). The highest yields of the

racemic chlorohydrins were obtained with 0.3-1 equivalents of base in the deprotonation step of the the starting materials **1**-**4**, 2 equivalents of epichlorohydrin, 12-26 h reaction time and 30 °C reaction temperature. The intermediate epoxides **1e**-**4e** were opened with lithium chloride and protonated with acetic acid. Recently, we have reduced the amounts of acetic acid from 10 to five eqivalents giving the same yields of the chlorohydrins. Kinetic resolutions of the racemic halohydrins were performed in different solvents with lipase B from *Candida antarctica* from SyncoZymes, Co. Ltd, (Shanghai, China) and vinyl butanoate as the acyl donor. Amination of the *R*-chlorohydrins (*R*)-**1a** and (*R*)-**3a**-**4a** gave the *S*-β-blockers with retention or increased *ee*. Due to the low *ee* (92%) amination of (*R*)-**2a** was not performed. Previously, we have published the synthesis of the building block for (*S*)-atenolol in > 98% *ee* by a similar protocol [5].



Scheme 1. Building blocks (*R*)-**1a**-**4a** synthesized in 92-97% *ee* for use in synthesis of the *S*-enantiomers of the β-blockers practolol, pindolol and derivatives of carteolol ((*S*)-**1c**-**4c**).

Analysis of the reaction mixtures from the syntheses of **1a**-**4a** on LC-MS showed that the most abundant by-products in these reactions were the dimers **1d**-**4d** (Scheme 1) of the deprotonated starting materials **1**-**4**. In order to assure full conversion of the starting materials and avoid formation of the dimer by-products in the syntheses concentrations of base and 2-(chloromethyl)oxirane (epichlorohydrin), reaction time and temperature have been varied. When high concentration of base was used, the intramolecular cyclization of the anions of **1a**-**4a** was observed to boost by increased reaction time if not the acid and lithium chloride was added immediately after full conversion of the starting materials. Investigations of reaction conditions in synthesis of the racemic practolol precursor **1a** is shown in Table 1. When 0.5-1.0 equivalents of sodium hydroxide dissolved in water was used in the reaction of **1** with epichlorohydrin with a reaction temperature of 80°C stirring for 24 h only the dimer *N*,*N*-(((2-hydroxypropane-1,3-diyl)bis(oxy))-bis(4,1-phenylene))diacetamide (**1d**) was obtained in addition to a small fraction of the epoxide **1e** (Table 1). Dimer **1d** was characterized by NMR-, MS- and IR-analyses. The chemical shifts for **1d** were assigned using ^{1}H-NMR-, ^{13}C-NMR-, COSY-, HSQC and HMBC-spectra. The analyses were performed on a 600 MHz NMR-instrument from Bruker, Germany, with deuterated dimethyl sulfoxide as solvent.

The strategy in order to avoid the formation of the dimers **1d**-**4d** and also to generate a high ratio of halohydrin to epoxide was to use only 0.3 equivalents of base. The chlorohydrins **1a**-**4a** were synthesized in 59-77% yield, see Table 3.

Table 1. A reaction temperature of 80°C and 0.5-1.0 eq of NaOH dissolved in water favored the formation of the dimer **1d** in the reaction of **1** with 2 eq of epichlorohydrin (Scheme 1).

Base	Equivalent	Rx temp.	Rx. time	1a (%)	1e (%)	1d (%)
NaOH	0.5	80 °C	24	0	3	95
NaOH	1.0	80 °C	16	0.4	0.3	99
NaOH	5.0	80 °C	16	1	11	6
NaOH	1.0	0 °C	32	-	-	-
K_2CO_3	1.0	r.t.	27	-	2	-
K_2CO_3	1.0	60 °C	27	2	12	72

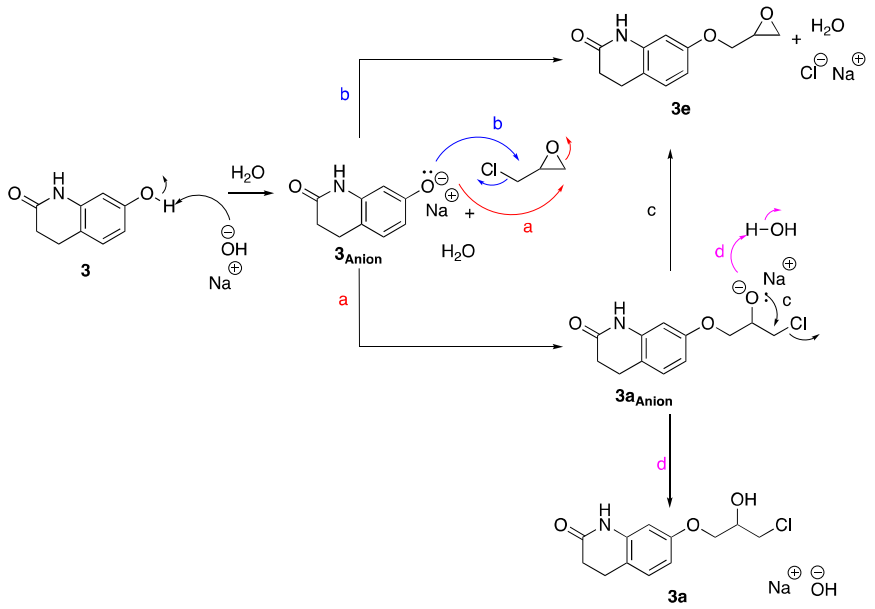
The starting material 5-(3-chloro-2-hydroxypropoxy)-3,4-dihydroquinolin-2(1*H*)-one (**4**) for the synthesis of carteolol is quite expensive, so we wanted to investigate similar reactions of 7-(3-chloro-2-hydroxypropoxy)-3,4-dihydroquinolin-2(1*H*)-one (**3**) as a model substrate.

Table 2 entry 3 shows that the highest relative rate of the formation of halohydrin **1a** over the formation of the epoxide **1e** is obtained with one eq. of base.

Table 2. Synthesis of N-(4-(oxiran-2-ylmethoxy)phenyl)acetamide, **1a**, and N-(4-(3-chloro-2-hydroxypropoxy)phenyl)acetamide, **1e**, from paracetamol, **1**, with epichlorohydrin an NaOH in rt. The table shows reaction conditions, equivalents and starting amounts of Base (mmol), and relative rate of **1a** and **1e** in the product mixture, calculated from HPLC chromatograms on Eclipse XDB-C18-column and gradient program (H_2O:MeCN 90:10 - H_2O:MeCN 75:25 over 20 min, 0.5 mL/min flow).

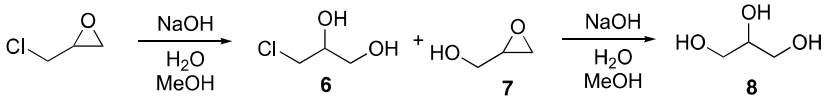
Entry	Paracetamol (mmol)	Equiv. NaOH	NaOH (mmol)	H_2O (mL)	Rx. time (h)	Appearance of rx mixture	1a (%) (t_R=11.0 min)	1e (%) (t_R=13.0 min)
1	6.62	0.1	0.661	1.9	48	Pink visc. liq.	29	38
2	6.62	0.5	3.31	4.7	8	Pink visc. liq.	37	43
3	6.62	1	6.62	5.8	7.5	White solid	81	12
4	19.8	1	19.8	10.0	5	White solid	65	1
5	3.31	2	6.62	4.7	7	White solid	39	0
6	0.662	10	6.62	5.0	18	Brown visc. liq	0	3

We saw the same trend in the synthesis of **2a**/**2e.** However, in the synthesis of **3a**/**3e** and **4a/4e** 0.3 eq of base gave the highest yield. When catalytic amounts of base are used the anions of the halohydrins formed will likely deprotonate a water molecule which regenerates the base for new deprotonations of the starting materials. A plausible mechanism for regeneration of the base in these reactions is shown for the reaction of 7-(3-chloro-2-hydroxypropoxy)-3,4-dihydroquinolin-2(1*H*)-one (**3**, Scheme 2). Addition of lithium chloride and acetic acid before any work up gave higher yields than when the reactions were worked up after the nucleophilic attack of epichlorohydrin in the first step.



Scheme 2. Mechanism for base catalyzed deprotonation of starting material **3** in the synthesis of halohydrin **3a** and the intermediate epoxide **3e** used in the synthesis of semi-carteolol, (*S*)-**3c**.

As the hydroxide ion also may attack epichlorohydrin directly both on carbon 1 in the oxirane and on the α-carbon the by-products 2-(chloromethyl)oxirane (**6**), 3-chloropropane-1,2-diol (**7**) and propane-1,2,3-triol (**8**) may also be formed, see Scheme 3. All impurities were removed by flash chromatography.



Scheme 3. 2-(Chloromethyl)oxirane (**6**), 3-chloropropane-1,2-diol (**7**) and propane-1,2,3-triol (**8**) may be formed in side reactions between epichlorohydrin and sodium hydroxide found in previous studies of these reactions in our group. Small amounts of 1,-3-dichloro-2-propanol has been found from GC-MS analyses in the synthesis of **2a**.

Kinetic resolutions of **1a**-**4a** have been performed catalyzed by CALB from Synco-Zymes Co LTD, Shanghai, China, with moderate to high *E*-values (calculated by *E&K Calculator*, 2.1b0 PPC) [22], giving moderate to high *ee*-values of the chiral building blocks. Du to the relative low *ee* of (*R*)-**2a** we did not proceed with the synthesis of pindolol enantiomer, however, we would have expected to retain the *ee* from the amination of (*R*)-**2a** giving (*S*)-pindolol in 92% *ee*.

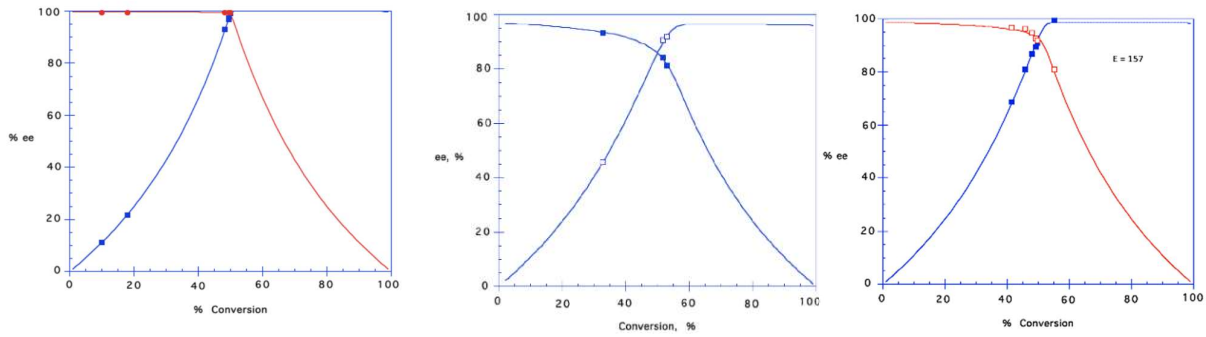


Figure 1. Graphical illustration of reaction progress of kinetic resolution at different degrees of conversion. Left panel: **1a** ee_P (red filled squares) and ee_S (blue filled squares) *E*-value >200. Middle panel: kinetic resolution of **2a** ee_P (filled squares) and ee_S (open squares) *E*-value 66. Right panel: **3a** ee_P (open red squares) and ee_S (filled blue squares) *E*-value 157. All three reactions used CALB from SyncoZymes as catalyst and vinyl butanoate as acyl donor in different solvents. All reactions were peromed with 30ºC reaction temp. *E*-values calculated from *E&K Calculator* 2.1b0 PPC [22].

In the amination reactions of (*R*)-**1a** and (*R*)-**3a** the *ee* was retained and (*S*)-practolol ((*S*)-**1c**) and and (*S*)-semi-carteolol ((*S*)-**3c**) were produced in 96 and 97% *ee*, respectively. (*R*)-**4**a was obtained in 99% *ee*, and (*R*)-4c was obtained in 99% ee from amination of (*R*)-**4a**. (Table 3). Reaction times of the kinetic resolutions varied by varied amounts of lipase added, however, 12h should be a proper reaction time.

We have noticed that other research groups have reported data for synthesis of enantiopure practolol and pindolol [12, 15]. Reaction time for the enzymatic hydrolyses of the acetic ester 1-(1H-indol-4-yloxy)-3-chloro-2-propanol reported by Lima *et al.* varied from 12-25 h and the authors reported an *E*-value of 30 in the hydrolytic kinetic resolution of the racemic acetylester of **2a** using Novozym® 435 [15]. By using lipase from *Pseudomonas fluorescens* in acetylation of racemic **2a**, an *E*-value of 11 was obtained, with an ee_S of the chlorohydrin **2a** with 72% ee_S and of 69% ee_P for 51% conversion, 24 h reaction time with 40ºC. However, there is some misunderstandings of stereochemistry in Lima´s report. The authors report that hydrolysis of the racemic acetic ester and transesterification reaction of the chlorohydrin **2a** give enantiomers with opposite stereochemistry. The product and the remaining alcohol will have the same stereochemistry in hydrolysis of the acetyl ester and transesterification of the chlorohydrin **2a**. Hydrolysis of the ester enantiomer from the hydrolysis of racemic ester should not be the *R*-acetic ester of **2a**, but the *S*-ester. We claim that they have produced (*S*)-**2a** in 97% *ee* instead of (*R*)-**2a** in their hydrolysis of the acetic ester of 1-((1H-indol-4-yl)oxy)-3-chloropropan-2-ol. The authors must have used the *S*-halohydrin in the synthesis and would then have achieved (*R*)-pindolol. The optical rotation value is not reported. In our project we used CALB from SyncoZymes as catalyst and obtained an *E*-value of 66 in the esterification of **2a.** At 53% conversion (24h) ee_S and ee_P values were 92% and 81% respectively.

Table 3. *E*-values, *ee*-values and yields of racemic building blocks **1**-**4** and of *R*-alcohols (*R*)-**1a**-**4a** and the drugs (*S*)-**1c** and **3c** from the reactions of **1**-**4** with vinyl butanoate as acyl donor to produce the butanoates (*S*)-**1b-4b** leaving the *R*-enantiomers unreacted. Different solvents were used and the kinetic resolutions were catalyzed by lipase B from *Candida antarctica* from SyncoZymes Co. Ltd. (Shanghai, China). Optical rotations $[\alpha]_D^T$ were determined at 20-23°C in different solvents with c=1, for additional parameters, see Experimental section.

Halohydrin	Yield	*E*-value	Ester, *ee*, yield, %	$[\alpha]_D^{20}$(solvent)	Halohydrin, *ee*, yield, %	$[\alpha]_D^T$(solvent)	Drug, *ee*, yield, %	$[\alpha]_D^{20}$(solvent)
1a	68	51	(*S*)-**1b**, 84, 47	+16.3º (c 1.1 MeCN, 23ºC)	(*R*)-**1a**, 97, 38 (*S*)-**1a**, 81, 26	– 1.0º (*i*-PrOH) + 11.99 º (*i*-PrOH)	(*S*)-**1c**, 96, 16	– 3.998º (EtOH)
2a	59	66			(*R*)-**2a**, 92		(*S*)-**2c**	
3a	77	157	(*S*)-**3b**, 86, 51	+8.0ºº(MeOH)	(*R*)-**3a**, 96, 34	−9.9º (MeOH)	(*S*)-**3c**, 97, 82	−16.0º (MeOH).
4a	61	100			(*R*)-**4a**, 98		(*S*)-4**c**, 98	

3. Materials and methods

All chemicals used in this project are commercially available, of analytical grade and was purchased from Sigma-Aldrich Norway (Oslo, Norway) or vwr Norway (Oslo, Norway). HPLC grade of solvents were used for the HPLC-analyses. Dry MeCN was acquired from a solvent purifier, MBraun MD-SPS800 (München, Germany) and stored in a flask containing molecular sieves (4Å).

3.1. Enzymes

Candida antarctica Lipase B (CALB) (activity ≥ 10000 PLU/g, lot#20170315) immobilized at high hydrophobic macro porous resin, produced in fermentation with genetically modified *Pichia pastoris*. Gift from SyncoZymes Co, Ltd. (Shanghai, China).

3.2. Chromatographic analyses

All analyses were performed on an Agilent HPLC 1100. Manual injector (Rheodyne 77245i/Agilent 10 µl loop), and a variable wavelength detector (VWD) set to 254 nm.).

3.2.1. Achiral HPLC

Separation was performed on a XDB C18-column (250 × 4.6 mm ID, 5 µm particle size, 80Å, Phenomenex, Oslo, Norway).

Dimer **1d**: eluent gradient: H_2O:acetonitrile (90:10) - H_2O:acetonitrile (75:15) over 20 min: t_R 15.71 min.

3.2.2. Chiral HPLC

Separation of enantiomers was performed on a Chiralcel OD-H column (250 × 4.6 mm ID, 5 µm particle size, Daicel, Chiral Technologies Europe, Gonthier d´Andernach, Illkirch, France). Baseline separation was obtained if not R_s is given. Chlorohydrin **1a:** t_R (*S*)-**1a**= 31.1 min, t_R (*R*)-**1a**)= 38.8 min. Butanoate ester **1b**: t_R(*S*)-**1b** = 18.9 min and (*R*)-**1b** 23.7 min, eluent hexane: *i*-PrOH, eluent: hexane: *i*-PrOH (83:17), 1 mL/min flow, R_s= 12.05 and t_R (*S*)-**1b** = 24.8 min and (*R*)-**1b** 31.6 min, eluent hexane: *i*-PrOH (85:15), R_s= 8.91. Practolol **1c,** t_R(*R*)-**1c**) =13.6 min, t_R(*S*)-**1c**). =17.2 min, eluent hexane: *i*-PrOH (83:17), R_s=6.17 Chlorohydrin **2a**: t_R (*S*)**-2a** = 50.7 min, t_R (*R*)**-2a** = 22.5 min, butanoate ester **2b:** t_R (*S*)-**2b** = 14.0 min, t_R (*R*)-**2b** = 14.7 min, eluent: hexane: *i*-PrOH (80:20), 1 mL/min flow. Chlorohydrin **3a:** t_R (*S*)-**3a** = 20.10 min, t_R(*R*)-**3a** = 25.83 min. Eluent: *n*-hexane:*i*-PrOH:DEA (60:40:0.4), Butanoate ester **3b**: t_R (*S*)-**3b** = 56.30 min, t_R (*R*)-**3b** = 63.47 min. Eluent: *n*-hexane:*i*-PrOH:DEA 90:10:0.4. 0.4 mL/min flow. Chlorohydrin **4a:** t_R (*S*)-**4a** = 63.43 min, t_R(*R*)-**4a** = 68.68 min. Eluent: *n*-hexane:EtOH:DEA:TFA (90:10:0.1), 0.4 mL/min flow.Rs=1.62. Butanoate ester **4b**: t_R (*S*)-**4b** = 56.30 min, t_R (*R*)-**4b** = 63.47 min. Eluent: *n*-hexane:*i*-PrOH:DEA 90:10:0.4.

3.3. TLC-analyses and column chromatography

TLC-analyses were performed on Merck silica 60 F_{254} and detection with UV at λ = 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).

A New Brunswick G24 Environmental Incubator Shaker (New Brunswick co. Inc., Edison, New Jersey, USA) was used for enzymatic reactions.

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 (Bruker, Rheinstetten, Germany). Chemical shifts are in ppm rel. to TMS and coupling constants are in Hertz (Hz). Infrared spectroscopy was performed at a Nexus FT-IR instrument (Madison, WI, USA). Exact masses were analysed with a Synapt G2-S Q-TOF mass spectrometer from Waters™. Ionization of samples was done with an ASAP probe (APCI), and calculation of exact masses and spectra processing was performed with Waters™ Software (Masslynxs V4.1 SCN871).

3.5. Optical rotations

Optical rotations were measured on an Anton Paar (MCP 5100) polarimeter with a 2.5 cm long cell. (Oslo, Norway). The analyses were performed at 20-23°C and the samples were dissolved in different solvents. Wavelength of the light was 589 nm (D).

3.6. Absolute configurations

Absolute configurations of (*S*)-**1c** and (*S*)-**4c** were determined by comparing the optical rotation with previously reported data. Optical rotation values of (*R*)-**1a**, (*R*)-**3a**, (*S*)-**3c** and (*R*)-**4a** have not been reported previously and were determined by the enantioselectivity of CALB which we have reported previously [20, 21].

3.7. Synthesis protocols

N-(4-(3-chloro-2-hydroxypropoxy)phenyl)acetamide, 1a

N-(4-Hydroxyphenyl)acetamide (paracetamol, 151.163 g/mol, 1.998 g, 13.22 mmol) was dissolved in NaOH solution (0.5 eq), and 2-(chloromethyl)oxirane (epichlorohydrin) (26.44 mmol, 2 equiv.) was added drop wise under stirring. The reaction mixture was stirred at rt until TLC (CH_2CH_2: MeOH, 10:1) showed full conversion of paracetamol. The reaction mixture was filtrated and washed with MeCN or dist. H_2O. The filtrate was extracted with EtOAc, and the organic layer was dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The product mixture was analyzed by HPLC with EclipseXDB C18-column and gradient (H_2O:MeCN (90:10) - H_2O:MeCN (75:25) over 20 minutes, 0.5 mL/min flow) showing both the chlorohydrin N-(4-(3-chloro-2-hydroxypropoxy)phenyl)acetamide, **1a**, and the epoxide N-(4-(oxiran-2-ylmethoxy)phenyl)acetamide, **1e**. The product mixture was reacted further without purification. The amounts of reagents in step 2 are calculated from the assumption that the starting material contains only N-(4-(oxiran-2-ylmethoxy)phenyl)acetamide, **1e**, even if the starting material also contained N-(4-(3-chloro-2- hydroxypropoxy)phenyl)acetamide, **1a**. The mixture of **1a/1e** (0.681 g, 3.29 mmol) was dissolved in MeCN (10 mL), and LiCl (0.912 g, 21.5 mmol) and AcOH (3.0 mL, 53.1 mmol) was added. The reaction mixture was stirred at rt for 26h and TLC (CH_2CH_2:MeOH, 10:1) showed only the chlorohydrin **1a**, R_f = 0.50. The reaction was stopped by adding Na_2CO_3 until reaching neutral pH. The precipitated salt was filtrated off. The reaction mixture was then extracted with EtOAc and washed with satd. NaCl solution. The organic layer was dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. A yellow/brown viscous liquid was collected, which was purified with flash chromatography (CH_2CH_2:MeOH, 10:1, v/v). After purification the product **1a** was collected as a white solid (0.543 g, 2.24 mmol) in 68% yield in >99% purity. (HPLC, t_R =13.4 min). TLC (CH_2CH_2:MeOH 10:1) R_f =0.43 for N-(4-(3-chloro-2-hydroxypropoxy)phenyl) acetamide. ^{1}H NMR (400MHz, $DMSO_{d6}$): δ 9.78 (s, 1H, NH), 7.48-7.46 (d, 2H, AR-H, *J*=10.2 Hz), 6.89-6.87 (d, 2H, Ar-H, *J*=9.6 Hz), 5.53-5.52 (d, 1H, OH, *J*=5.4 Hz), 4.03-3.99 (sextet, 1H, CH, *J*=5.3 Hz), 3.93-3.92 (d, 2H, CH_2, *J*=5.6 Hz), 3.76-3.72 (dd, 1H, CH_2, J_1=5.4 Hz, J_2=11.8 Hz), 3.68-3.64 (dd, 1H, CH_2, J_1=5.4 Hz, J_2=10.8 Hz), 2.00 (s, 3H, CH_3). ^{13}C NMR (400MHz, $DMSO_{d6}$): δ 168.1 (C=O), 154.6 (Ar-C-O), 133.2 (Ar-C-N), 120.9 (2 x Ar-C), 114.9 (2 x Ar-C), 69.6 (CH_2), 69.1 (CH), 47.2 (CH_2), 24.3 (CH_3). HRMS (TOF-ASAP$^+$): $[M+H]^+$=244.0739 *m*/*z* (calc. mass: 244.0740, $C_{11}H_{15}NO_3Cl$).

1. -((1H-indol-4-yl)oxy)-3-chloropropan-2-ol (**2a**) [15]

1H-Indol-4-ol (0.51 g, 3.80 mmol) was dissolved in 1,4-dioxane (3 mL) and NaOH (0.16 g, 3.93 mmol) was dissolved in water (5 mL) and added. Epichlorohydrin (2.98 mL, 38 mmol) was added. The mixture was stirred at rt for 5 h until TLC showed full conversion of starting material (CH_2Cl_2, R_f=0.21). The product was extracted using CH_2Cl_2 (50 mL) and washed with EtOAc (30 mL x 3) and water (30 mL x 3). The CH_2Cl_2-phase was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure, yielding 0,48 g of a mixture of 1-((1H-indol-4-yl)oxy)-3-chloropropan-2-ol (**2a**) and 4-(oxiran-2-ylmethoxy)-1H-indole (**2e**) as a brown oil.

A mixture of **2a**/**2e** (0.48 g, 2.56 mmol) was dissolved in THF (8 mL). AcOH (1.46 mL, 25.6 mmol) and LiCl (0.22 g, 5.12 mmol) were added. The mixture was stirred at rt for 72 h. $NaCO_3$ was added until neutral pH. The product was extracted with CH_2Cl_2 (50 mL) and washed with satd. NaCl solution (30 mL x 3). The CH_2Cl_2 phase was then dried over anhydrous $MgSO_4$ and evaporated, yielding 0,5701 g of 1-((1H-indol-4-yl)oxy)-3-chloropropan-2-ol (**2a**). After purification by flash chromatography with CH_2Cl_2 as eluent, the product was obtained as a slightly yellow oil. Spectroscopic data for **2a:** ^{1}H NMR (400 MHz, $DMSO_{d6}$) δ 11.07 (s, 1H, H12), 7.22 (t, 1H), 7.02 (m, 2H), 6.47 (t, 1H), 6.49 (dd, 1H), 5.5 (s, 1H), 4.09 (m, 3H), 3.84 (dd, 1H), 3.75 (dd, 1H). ^{13}C NMR (400 MHz, $DMSO_{d6}$) δ 152.4 (C4), 137.9 (C8), 124.1 (C11), 122.5 (C6), 118.9 (C9), 105.6 (C7), 100.4 (C5), 98.7 (C10), 69.3 (C3), 69.2 (C2), 47.5 (C1). HRMS (TOF ASAP$^+$):$[M+H]^+$ 226.0632 *m/z*

1. -((1H-indol-4-yl)oxy)-3-chloropropan-2-yl butanoate (**2b**)

1-((1H-Indol-4-yl)oxy)-3-chloropropan-2-ol (**2a**) (0.08 g, 0.37 mmol) and butyric anhydride (0.075 mL, 0.46 mmol) were added to pyridine (0.05 mL, 0.62 mmol). The mixture was stirred for 24h at rt. Extraction was performed with hexane and CH_2Cl_2 and washed with satd. NaCl solution. The organic phase was dried over anhydrous $MgSO_4$ and evaporated, yielding 0,08 g of a mixture of **2a** (84.6%) and **2b** (11.0%). Separation by flash-chromatography using CH_2Cl_2 as eluent yielded 1% of **2b** (0.80 mg, 0.003 mmol). Spectroscopic data for **2b**: ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 8.22 (s, 1H, H12), 7.03-7.16 (m, 3H, H11/H7/H6), 6.64 (m, 1H, H10), 6.56 (dd, 1H, H5), 4.19-4.37 (m, 3H, H3/H2), 3.75-3.90 (m, 2H, H1), 2.37 (td, 2H, H14), 1.69 (sex, 2H, H15), 0.97 (t, 3H, C16). ^{13}C NMR (400 MHz, $CDCl_3$) δ_C: 151.86 (C4), 137.38 (C8), 122.93 (C11), 122.77 (C6), 118.68 (C9), 105.20 (C7), 101.01 (C5), 99.67 (C10), 70.05 (C3), 68.64 (C2), 46.19 (C1), 36.40 (C14), 18.44 (C15), 13.61 (C16).

7. -(3-Chloro-2-hydroxypropoxy)-3,4-dihydroquinolin-2(1H)-one (**3a**) [23]

To a solution of **3** (1.6541 g, 10.1 mmol) in MeOH (30 mL), NaOH-solution (0.17 M, 30 mL) was added. Epichlorohydrin (1.565 ml, 20.0 mmol) was added dropwise to the reaction mixture which was then stirred at rt for 24 h. TLC ($CHCl_3$:CH_2Cl_2:EtOH, 10:9:1 showed full conversion of **3a** with two products: R_f (**3e**) = 0.31, R_f (**3a**) = 0.41. Insoluble by-products filtered off. The filtrate was extracted with CH_2Cl_2 (20 mL × 3). The organic phase was washed with satd. NaCl solution (10 mL × 2), dried over anhydrous $MgSO_4$, filtered before the solvent was removed under reduced pressure. This resulted in white crystals and a yellow, highly viscous oil in a mixture. The mixture was recrystallized in EtOH to yield **3e** as white crystals (1.1074 g, 5.05 mmol, 50% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 2.55 (m, 2H, CH_2), 2.74; 2.88 (dd, 2H, C**H**$_2$-O-CH), 2.88 (m, 2H, CH_2), 3.84; 4.27 (m, 2 H, -O-C**H**$_2$-CHOCH_2), 6.49 (m, 1H, Ar-H), 6.59 (m, 1H, Ar-H), 7.08 (m. 1H, Ar-H). ^{13}C NMR (400 MHz, CD_3OD) δ: 24.0, 30.5, 43.5, 49.8, 68.9, 102.0, 108.5, 116.4, 128.3, 138.4, 158.2, 172.7.

LiCl (1.0440g, 24.6 mmol) and AcOH (2.810 ml, 49.1 mmol) were added to a solution of 3**a**/**3e** (1.0766 g, 4.91 mmol) in MeCN (10 mL). The reaction was stirred at rt and TLC showed full conversion of the starting material after 24h. The reaction mixture was extracted with CH_2Cl_2 (3 × 20 ml), dried over anhydrous $MgSO_4$ and the solvent was removed under reduced pressure. **3a** was obtained as white crystals (0.9647 g, 3.77 mmol,

77% yield). ^{1}H NMR (400 MHz, CD_3OD) δ: 2.55 (q, 2H, CH_2), 2.86 (m, 2H, CH_2), 3.69;3.77 (m, 2H, CH_2-Cl), 4.06 (m, 2H, O-C**H**$_2$-CHOH), 4.13 (m, 1H, C**H**OH), 6.51 (m, 1H, Ar-H), 6.60 (m, 1H, Ar-H), 7.09 (m, 1H, Ar-H). ^{13}C NMR (400 MHz, CD_3OD) δ: 24.0, 30.5, 45.4, 68.9, 69.6, 102.0, 108.5, 116.4, 128.3, 138.4, 158.2, 172.7

5. -(3-Chloro-2-hydroxypropoxy)-3,4-dihydroquinolin-2(1H)-one (**4a**)

Epichlorohydrin (0.134 ml, 1.7 mmol) was added to a solution of **4** (0.1665g, 1.0 mmol) in H_2O (0.585 mL) and DMSO (0.375 mL). NaOH (0.090 ml, 9.5M) was added to the reaction mixture. The solution was stirred at rt for 24 hours where **4a** and **4e** slowly precipitated from the solution. TLC (*n*-hexane:*i*-PrOH, 4:1) showed full conversion after 24h and the product mixture was filtered off yielding **4a** and **4e** (0.1505 g) as white crystals in a 1:4 ratio, determined by ^{1}H NMR. TLC ($CHCl_3$:Acetone 4:1, R_f(**4a**) = 0.32, R_f(**4e**) = 0.46). The mixture of **4e/4a** (1.0979 g, 5.01 mmol) was then dissolved in MeCN (5 mL). LiCl (1.0741 g, 25 mmol) and AcOH (2.865 mL, 50 mmol) was added and the reaction mixture was stirred for 24h when TLC showed full conversion of **4e**. Na_2CO_3-solution (pH 12) was added until the reaction mixture reached pH 7. The product was filtered off and **4a** (0.7807 g, 3.1 mmol) was obtained as white crystals in a global yield of 61%.^{1}H NMR (400 MHz, CD_3OD) δ 2.43 (t, 2H, CH_2), 2.86 (t, 2H, CH_2), 3.60-3.69 (m, 2H, CH_2-Cl), 3.98 (m, 2H, O-C**H**$_2$-CHOH), 4.05 (m, 1H, C**H**-OH), 6.41 (m, 1H, Ar-H), 6.56 (m, 1H, Ar-H), 7.01 (m, 1H, Ar-H). ^{13}C NMR (400 MHz, CD_3OD) δ 18.1, 29.6, 45.4, 69.0, 69.6, 106.3, 108.5, 111.9, 127.6, 138.6, 155.8, 172.5.

Practolol (N-(4-(2-hydroxy-3-(isopropylamino)propoxy)phenyl)acetamide),**1c**

Racemic N-(4-(3-chloro-2-hydroxypropoxy)phenyl)acetamide **1a** (0.220 g, 0.905 mmol) was dissolved in isopropylamine (0.519 mL, 6.33 mmol, 7.0 equiv.) and dist. H_2O (0.150 mL).The reaction mixture was stirred at rt for 48 h, and was concentrated under reduced pressure. The crude product was recrystallized with MeCN, and N-(4-(2-hydroxy-3-(isopropylamino)propoxy)phenyl)acetamide, **1c**, was collected as a white solid (0.115 g, 0.432 mmol, 48%). ^{1}H-NMR (400 MHz, $DMSO_{d6}$): δ 9.74 (s, 1H, NH), 7.45-7.43 (d, 2H, Ar-H-C), *J*=10.8 Hz), 6.85-6.83 (d, 2H, Ar-H-C, *J*=10.8 Hz), 4.92-4.90 (d, 1H, OH, *J* = 5.3 Hz), 3.89-3.86 (m, 1H, CH), 3.80-3.79 (t, 2H, CH_2, *J*=6.7 Hz), 2.73-2.62 (m, 2H, CH_2), 2.53-2.51 (m, 1H, CH), 1.99 (s, 3H, CH_3), 1.46 (s, 1H, NH), 0.96-0.94 (dd, 6H,2 x CH_3, J_1=2.2 Hz, J_2=6.2 Hz). ^{13}C-NMR (600MHz, DMSO $_{d6}$): δ 167.7 (C=O), 154.4 (Ar-C, 132.4 (Ar-C),120.4 (2 x Ar-C-H), 114.3 (2 x Ar-C-H), 70.9 (CH), 68.4 (CH_2), 50.0 (CH), 48.1 (CH_2), 23.7 (CH_3), 22.9 (2 x CH_3). HRMS (TOF-ASAP$^+$): $[M+H]^+$=267.1712 *m*/*z* (calc. mass: 267.1709, $C_{14}H_{23}N_2O_3$). IR (cm^{-1}, diluted): 3343 (m), 2969 (m), 1127 (m), 950 (s), 816 (m). MS (TOF-ASAP): $[M+H]^+$ = 267.1711m/z (calc. Mass [M+H]: 267.1709, $C_{14}H_{23}N_2O_3$).

3.8. Synthesis of enantiomers

Kinetic resolution of **1a**

Racemic *N*-(4-(3-chloro-2- hydroxypropoxy)phenyl)acetamide, **1a**, (0.543 g, 2.24 mmol) was dissolved in dry MeCN (60 mL). Vinyl butanoate (2.56 g, 22.4 mmol) and molecular sieve were added. The reaction was started by adding CALB (1.22 g) and stirred in an incubator shaker (30ºC, 200 rpm). Samples (150 µL) were collected regularly and concentrated and dissolved in *i*-PrOH before HPLC-analyses. After 26h the reaction was stopped by filtering off the enzyme and removal of the solvent under reduced pressure. The crude product was purified twice with flash chromatography (CH_2Cl_2:EtOAc, 1:1). (*R*)-**1a** was collected as a white solid (0.208 g, 0.852 mmol, yield 38%, *ee* =97%). $[\alpha]_D^{23} = -1.0^{o}$ (c 1.0 *i*-PrOH). (*S*)-1-(4-Acetamidophenoxy)-3-chloropropan-2-yl butanoate, (*S*)-**1b**, was collected as a brown viscous liquid (0.33 g, 1.05 mmol, yield 47%, *ee*=84%). $[\alpha]_D^{23} = +16.3^{o}$ (c 1.1 MeCN). (*S*)-*N*-(4-(3-chloro-2-hydroxypropoxy)phenyl)acetamide, (*S*)-**1a**, was obtained by hydrolysis of (*S*)-**1b** with CALB: The crude product was purified with flash chromatography (CH_2Cl_2:EtOAc 1:1) and (*S*)-**1a** was collected as a light brown solid (0.0475 g,

0.195 mmol, 26%, *ee*=81%) $[\alpha]_D^{20}$ = +11.99º (c 1.0 *i*-PrOH). The *E*-value was calculated by E&K calculator 2.1b0 PPC, *E*=55.

Kinetic resolution of **2a**

1-Chloro-3-(1H-indol-4-yloxy)-propan-2-ol (**2a**) (18.5 mg, 0.08 mmol) was dissolved in dry CH_2Cl_2 (3 mL) and molecular sieve was added. Vinyl butanoate (75 µL, 0.59 mmol) and immobilized CALB (36.8 mg) were added, and the reaction was stirred in the incubator shaker for 24h (30°C, 200 rpm) to reach 50% conversion. Samples (150 µL) were collected regularly for chiral HPLC-analysis. (*R*)-2a was obtained in 92% ee and the E-value = 66. NMR spectra were in accordance with the spectra from **2a**.

Kinetic resolution of **3a**

Chlorohydrin **3a** (0.7492 g, 2.93 mmol) was dissolved in dry MeCN (60 ml) and molecular sieve (4Å) was added. Vinyl butanoate (1.860 ml) was added to the solution and the reaction was started by adding CALB (1.3724 g) and placing the container in an incubator shaker (30°C, 200 rpm). The reaction was stopped after 24h by filtering off CALB and the molecular sieve. The solvent was removed under reduced pressure yielding ester (*S*)-**3a** and chlorohydrin (*R*)-**2a** in a mixture as a brown oil, which were separated on flash column chromatography (EtOAc:*n*-hexane:MeOH 7:12:1). Chlorohydrin (*R*)-**3a** was isolated as white crystals (0.2573 g, 34% yield). *ee* = 96%, $[\alpha]_D^{20} = -9.9$ (c = 1.0, MeOH). Ester (*S*)-**3b** was obtained as a yellow oil (0.4436 g, 51% yield, 91% purity). HPLC (Eluent: *n*-hexane:*i*-PrOH:DEA 90:10:0.4, t_R = 56.30 min (*S*), t_R = 63.47 min (*R*), *ee* = 86%). $[\alpha]_D^{20} = +8.0$ (c = 1.0, MeOH). ^{1}H NMR (*R*)-**3a** (600 MHz, CD_3OD) δ: 0.96 (td, 3H, -CH_3), 1.65 (sext, 2H, CH_2), 2.35 (td, 2H, CH_2), 2.53 (t, 2H, CH_2), 2.85 (t, 2H, CH_2), 3.83;3.86 (m, 2H, CH_2-Cl), 4.15 (d, 2H, O-C**H**$_2$-CHOH), 5.33 (p, 1H, C**H**-O-CO), 6.48 (m, 1H, Ar-H), 6.56 (m, 1H, Ar-H), 7.06 (m, 1H, Ar-H). ^{13}C NMR (600 MHz, CD_3OD) δ: 12.3, 16.1, 23.7, 30.1, 35.2, 48.2, 66.1, 70.8, 101.8, 108.2, 116.3, 128.1, 138.2, 157.6, 172.3, 175.8. *E*-value=157.

Kinetic resolution of **4a** -large scale

Chlorohydrin **4a** (0.7492 g, 2.93mmol) was dissolved in dry MeCN (60 mL) and molecular sieve (4Å) was added. Vinyl butanoate (1.860 mL) was added to the solution and the reaction was started by adding CALB (1.3724 g) and placing the container in an incubator shaker (30°C, 200 rpm). The reaction was stopped after 24 h by filtering off CALB and the molecular sieve. The solvent was removed under reduced pressure yielding ester (*S*)-**4b** and chlorohydrin (*R*)-**4a** in a mixture as a brown oil, which were separated on flash column chromatography (EtOAc:*n*-hexane:MeOH 7:12:1). *E*-value=100. NMR spectra were in accordance with the spectra from **4a**.

3.9. Enantiopure drug derivatives

(*S*)-practolol, (S)-N-(4-(2-hydroxy-3-(isopropylamino)propoxy)phenyl)acetamide, (S)-**1c** (*R*)-N-(4-(3-Chloro-2-hydroxypropoxy)phenyl)acetamide, (*R*)-**1a**, (0.175 g, 0.719 mmol) was dissolved in *i*-$PrNH_2$ (0.470 mL, 5.73 mmol, 8.0 equiv.) and dist. H_2O (0.075 mL). The reaction mixture was stirred at rt for 96h, and the solvent was removed under reduced pressure. The crude product was recrystallized in MeCN and (*S*)-N-(4-(2-hydroxy-3-(isopropylamino)propoxy)phenyl)acetamide ((*S*)-**1c**) was collected as a white solid (0.0313 g, 0.117 mmol, 16%, *ee*= 96%). Optical rotation of (*S*)-**1c**: $[\alpha]_D^{20}$= −3.998º (c 1.0 EtOH). mp: 124.7-124.9ºC (lit. 130-131ºC) [23].^{1}H NMR (600 MHz, $DMSO_{d6}$): δ 9.75 (s, 1H, NH), 7.45-7.44 (d, 2 x Ar-H, *J* =8.4 Hz), 6.85-6.84 (d, 2 x Ar-H, *J*=8.4 Hz), 4.95 (s, 1H, OH), 3.90-3.87 (m, 1H, CH), 3.81-3.80 (d, 2H, CH_2, J = 6.8 Hz), 2.72-2.65 (m, 2H, CH_2), 2.55-2.52 (m, 1H, CH), 2.00 (s, 3H, CH_3), 0.98-0.96 (dd, 6H, 2 x CH_3, J_1=2.0 Hz, J_2=6.3 Hz). ^{13}C NMR (600 MHz, $DMSO_{d6}$): δ 167.7 (-C=O), 154.4 (Ar-C), 132.5 (Ar-C), 120.4 (2 x Ar-C), 114.4 (2 x Ar-C), 70.9 (CH), 68.3 (CH_2), 49.9 (CH), 48.2 (CH_2), 23.7 (CH_3), 22.7 (2 x CH_3). IR (cm^{-1}, diluted): 3343

(m), 2969 (m), 1127 (m), 950 (s), 816 (m). HRMS (TOF-ASAP$^+$): $[M+H]^+$=267.1711 *m*/*z* (calc. mass $[M+H]^+$= 267.1709, $C_{14}H_{23}N_2O_3$).

(*S*)-7-(3-(tert-butylamino)-2-hydroxypropoxy)-3,4-dihydroquinolin-2(1H)-one (*S*)-**3c**

Chlorohydrin (*R*)-**3a** (0.2573, 1.0063 mmol) was dissolved in *t*-$BuNH_2$ and H_2O and stirred at rt for 8 hours. *t*-$BuNH_2$ and H_2O was removed under reduced pressure yielding the crude product as a mixture of a yellow oil and white crystals. The crude product was purified by flash column chromatography (EtOAc:*n*-hexane:MeOH:TEA 80:7:10:3), yielding (*S*)-**3c** as a yellow oil (0.2419 g, 82% yield) in 89% purity (NMR). *ee* = 97%. $[\alpha]_D^{20} = -16.0$ (c = 1.0, MeOH). ^{1}H NMR (600 MHz, CD_3OD) δ 1.18 (s, 9H, $C(CH_3)_3$), 2.54 (t, 2H, CH_2), 2.73-2.81 (m, 2H, C**H**$_2$-NH), 2.88 (t, 2H, CH_2), 3.95 (m, 2H, CH_2-O-Ar), 4.02 (m, 1H, C**H**-OH), 6.50 (m, 1H, Ar-H), 6.59 (m, 1H, Ar-H), 7.08 (m, 1H, Ar-H). ^{13}C NMR (600 MHz, CD_3OD) δ 24.2, 27.1, 30.7, 44.8, 50.4, 68.7, 70.8, 101.9, 108.4, 116.1, 128.1, 138.3, 158.3, 172.6.

4. Conclusion

The *S*-enantiomers of practolol and carteolol derivative have been produced with *ee*´s of >96% from the chlorohydrins with the same *ee* produced from kinetic resolutions catalyzed by CALB from SyncoZymes, China. The syntheses of the chlorohydrins **1a**-**4a** have been optimized with reduction of base, temperature and reaction time compared to previously reported methods giving moderate to high total yields. With use of catalytic amounts of base and shorter reaction time the formation of by-products was reduced.

Author Contributions: Investigation, writing, original draft preparation, E.E.J.; supervision and writing, review and editing, E.E.J.; investigation M.A.G., G.B.A., S.S.L, M.B.H., M.R.

Funding: This research received no external funding.

Acknowledgments: EEA project 18-COP-0041 GreenCAM is thanked for support, SyncoZymes Co LTD, Shanghai, China is thanked for gift of CALB.

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

References

[1]. Causes of death, Norway, **2019**, http://statistikkbank.fhi.no/dar/(27/02/2021).
[2]. Ponikowski, P., Anker, S. D., AlHabib, K. F., Cowie, M. R., Force, T. L., Hu, S., Jaarsma, T., Krum, H., Rastogi, V., Rohde, L. E., Samal, U. C., Shimokawa, H., Siswanto, B. B., Sliwa, K., Filippatos, G., "Heart failure: preventing disease and death worldwide", *ESC Heart Failure* **2014**, 1, 4–25.
[3]. Klouman, M., Åsberg, A., Wideröe, T.-E., "The blood pressure level in a Norwegian population - the significance of inheritance and lifestyle", *Tidsskr. Nor. Laegeforen*. **2011**, 131, 1185–1189.
[4]. Patrick, G. L., An introduction to medicinal chemistry, 5th ed., Oxford University Press, Oxford, **2013**, pp 814
[5]. Lund, I. T., Bøckmann, P. L., Jacobsen, E. E., "Highly enantioselective CALB catalyzed kinetic resolution of building blocks for β-blocker atenolol", *Tetrahedron* **2016**, 72, 7288–7292.
[6]. Jacobsen, E.E and Anthonsen. T. Single Enantiomers from Racemates. Lipase catalysed Kinetic Resolution of Secondary Alcohols. In situ Stereoinversion, *Trends in Organic Chemistry*, **2017**, *18*, 71-83
[7]. Blindheim, F. H., Hansen, M. B. Evjen, S. Zhu, W. Jacobsen, E. E. Chemo-Enzymatic Synthesis of Enantiopure Synthons as Precursors for (*R*)-Clenbuterol and other β-2 agonists. *Catalysts*, **2018**, 8 (11), 516 doi:10.3390/catal8110516
[8]. Andersen, S., Refsum, H., Tanum, L., "Kirale legemidler", *Tidsskr. Nor. Laegeforen*. **2003**, 123, 2055–2056.
[9]. Barrett, A. M., Carter, J., Fitzgerald, J. D., Hull, R., Le Count, D., "A new type of cardioselective adrenoceptive blocking drug", *Br. J. Pharmacol.* **1973**, 48.
[10]. Pribble, A. H., Conn, R. D., "The use of practolol in supraventricular arrhythmias associated with acute illnesses", *Am. J. Cardiol. Heart.* **1975**, 35, 645–650.
[11]. Kahn. G., Cardiac drug therapy, 8th ed., Humana Press, **2015**, pp 769
[12]. Mulik, S., Ghosh, S., Bhaumik, J., Banerjee, U. C., "Biocatalytic synthesis of (*S*)-Practolol, a selective β-blocker", *Biocatalysis* **2016**, 1, 130–140.
[13]. Ader, U., Schneider, M. P., "Enzyme assisted preparation of enantiomerically pure

β-adrenergic blockers III. Optically active chlorohydrin derivatives and their conversion",
Tetrahedron: Asymmetry **1992**, 3, 521–524.

[14]. Koch-Weser, J.; Frishman, W. H., Pindolol: A New β-Adrenoceptor Antagonist with Partial Agonist Activity. *N. Engl. J. Med.* **1983,** *308* (16), 940-944.

[15]. Lima, G. V.; Da Silva, M. R.; de Sousa Fonseca, T.; de Lima, L. B.; de Oliveira, M. D. C. F.; de Lemos, T. L. G.; Zampieri, D.; Dos Santos, J. C. S.; Rios, N. S.; Gonçalves, L. R. B.; Molinari, F.; de Mattos, M. C., Chemoenzymatic synthesis of (*S*)-Pindolol using lipases. *Appl. Catal. A, General* **2017,** *546*, 7-14.

[16]. Liu, Y.; Zhou, X.; Zhu, D.; Chen, J.; Qin, B.; Zhang, Y.; Wang, X.; Yang, D.; Meng, H.; Luo, Q.; Xie, P., Is pindolol augmentation effective in depressed patients resistant to selective serotonin reuptake inhibitors? A systematic review and meta-analysis. *Hum. psychopharmacol.* **2015,** *30* (3), 132-42.

[17]. Watson, P. G.; Barnett, M. F.; Parker, V.; Haybittle, J., A 7 year prospective comparative study of three topical β blockers in the management of primary open angle glaucoma. *Brit. J. Ophthalmol.* **2001,** *85* (8), 962.

[18]. Schuster, A. K.; Erb, C.; Hoffmann, E. M.; Dietlein, T.; Pfeiffer, N., The Diagnosis and Treatment of Glaucoma. *Dtsch Arztebl Int* **2020,** *117* (13), 225-234.

[19]. http://www.reseptregisteret.no/default.aspx (accessed 27.02.2021).

[20]. Jacobsen, E. E.; Hoff, B. H.; Anthonsen, T. Enantiopure derivatives of 1,2-alkanediols. Substrate requirements for lipase B from Candida antarctica. Chirality **2000**, 12, 654-659

[21]. Jacobsen, Elisabeth Egholm; Anthonsen, Thorleif; el- Behairy, Mohammed Farrag; Sundby, Eirik; Aboul-Enein, Mohamed Nabil; Attia, Mohamed Ibrahim; El-Azzouny, Aida Abd El-Sattar; Amin, Kamilia M.; Abdel-Rehim, Mohamed. Lipase Catalysed Kinetic Resolution of Stiripentol. *Int. J. Chem*. 2012, *4* (1) 7-13

[22]. Anthonsen, H.W.; Hoff, B.H.; Anthonsen, T. Calculation of enantiomer ratio and equilibrium constants in biocatalytic pingpong bi-bi resolutions, *Tetrahedron: Asymmetry* **1996**, *7*, 2633-2638. DOI: https://doi.org/10.1016/0957-4166(96)00338-2

[23]. Manghisi, E. P., B.; Salmibeni, A. A process for the preparation of 5-(3-tert-butylamino-2-hydroxypropoxy)-3,4-dihydrocarbostyryl. EP0579096 (A2), 19.01.**1994**

[24]. Danilewicz, J. C. and Kemp J. E. G. Absolute Configuration by Asymmetric Synthesis of (+)-1-(4-Acetamidophenoxy)-3-(isopropylamino)-propan-2-ol (Practolol) *J. Med. Chem.* **1973**, Vol. 16, No. 2 168-169