Lipase- and Base Catalyzed Synthesis of Enantiopure β-Blocker (*S*)-Atenolol

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Article

Lipase- and Base Catalyzed Synthesis of Enantiopure β-Blocker (*S*)-Atenolol

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Abstract: (*S*)-Atenolol ((*S*)-2-(4-(2-Hydroxy-3-(isopropylamino)propoxy)phenyl)acetamide) has been synthesized in >99% *ee* with use of *Candida antarctica* lipase B from Syncozymes (Shanghai, China), in a kinetic resolution of the corresponding racemic chlorohydrin. Deprotonation of the starting phenol building block was base catalyzed. The enantiopurity of the chlorohydrin building block remained unchanged upon subsequent amination. All four steps in the synthesis protocol have been optimized compared to previously reported methods, which makes this new protocol more sustainable and in accordance with green chemistry principles compared to previously reported protocols.

Keywords: Enantiopure (*S*)-atenolol; *Candida antarctica* lipase B; base catalysis; Chiralcel OD-H column

1. Introduction

The American Heart Association reported in March 2019 their update on heart diseases and stroke statistics. The report states that high blood pressure concerned 46% of the total population at ages 20 years and older in the United States between 2013-2016. It was the cause of death for 82,735 Americans in 2016 and costed the American society approximately $ 55.9 billion in the period 2014-2015 [1]. In 2018, cardiovascular treatment made up 4.9% of the total pharmaceutical marked in Norway, which corresponded to 1,17 million Norwegian kroner [2]. A class of drugs that have been used in the treatment of both cardiovascular and non-cardiovascular diseases are the β-adrenergic blocking agents (beta-blockers). Approximately 300,000 patients in Norway use beta-blockers [3]. Worldwide, the use of beta-blockers increase year by year and the sales are estimated to account for 13,684 million USD by 2030 [4].

The highly polar cardio selective β1-antagonist atenolol is selective towards β1-receptors found in the heart. This drug is used in the treatment of hypertension, angina pectoris and arrythmia. Atenolol is one of the most widely used beta-blockers clinically and is often used as a reference drug for comparisons with other antihypertensives. The drug might however be even more effective in preventing myocardial infarction [5].

Atenolol is manufactured with enantiomerically pure active pharmaceutical ingredient (API) as Atpure® by Emcure Pharmaceuticals, (Pune, India) and with racemic API under the names Tenormin®, Mylan® and others. The eudismic ratio is 46 in favour of (*S*)- to (*R*)-atenolol [6], and studies in rats show that the *R*-enantiomer has no effect. While the racemic drug causes a lowering of the heart rate, this side effect is not observed with enantiopure (S)-atenolol [7].

Several synthesis protocols for producing enantiopure (*S*)-atenolol have been published. Emcure Pharmaceuticals uses enantiopure epichlorohydrin to produce enantiopure (*S*)-atenolol from deprotonated 2-(4-hydroxyphenyl)acetamide. The specific rotation of the final drug (*S*)-atenolol of $[\alpha]_D^{25}$= −17.1 (1.0, 1N HCl) is reported [8]. Dwivedee *et al.* also started with deprotonation of 2-(4-hydroxyphenyl)acetamide gaining only the epoxide 2-(4-(2-oxiran-2-ylmethoxy)phenyl)benzeneacetamide. Reaction of this epoxide with acetyl chloride in methanol gave the racemic 4-(3-chloro-2 hydroxypropoxy)benzeneacetamide, which was resolved by several lipase

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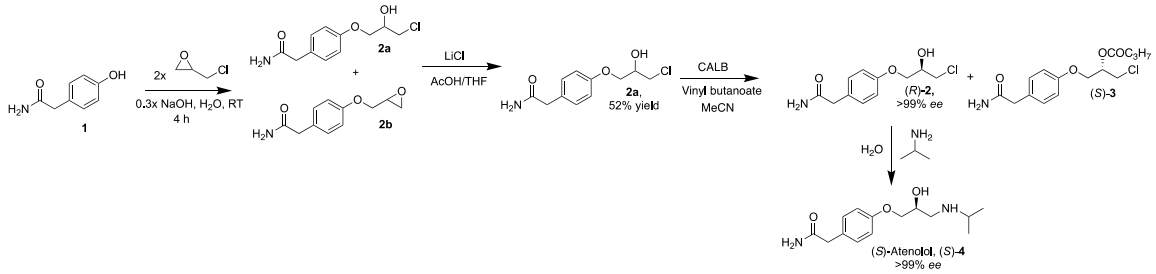
preparations and vinyl acetate as the acyl donor. The authors claim to have formed (*S*)-atenolol from (*S*)-4-(3-chloro-2-hydroxypropoxy)benzeneacetamide, which is not possible according to their reaction conditions [9]. To our knowledge, addition of isopropylamine in water to (*S*)-4-(3-chloro-2-hydroxypropoxy)benzeneacetamide leads to (*R*)-atenolol. The authors do not report any specific rotation of their (claimed) (*S*)-atenolol product, nor of their enantiopure building blocks. Agustian *et al.* present a similar study of (*S*)-atenolol synthesis using several lipase preparations to resolve the racemic 4-(3-chloro-2 hydroxypropoxy)benzeneacetamide [10]. The authors give no evidence of the absolute configuration of the product, nor any yields or evidence of the enantiomeric excess (*ee*) of the product. Sikora *et al.* reported in 2020 the kinetic resolution of racemic atenolol catalyzed by lipase from *Candida rugosa* with isopropenyl acetate as the acyl donor, giving the acetate of (*S*)-atenolol in 94% *ee*. The authors give no evidence of the absolute configuration of this acetate, nor of the unreacted (*R*)-atenolol [11,12]. The authors have previously published several articles of lipase catalyzed kinetic resolution of racemic atenolol with the amide of (*S*)-atenolol presented as the acetate [13-15]. (*S*)-Atenolol has been synthesized in 98% *ee* in a seven step method using Jacobsen's catalyst ((*R*,*R*)-salen Co(III)OAc) [16], and in 94% *ee* through kinetic resolution of racemic atenolol using lipase from *Pseudomonas cepacia* [17]. We have produced the enantiopure building block (*R*)-4-(3-chloro-2 hydroxypropoxy)benzeneacetamide starting with a deprotonation of 2-(4-hydroxyphenyl)acetamide with sodium hydroxide, and by using lipase B from *Candida antarctica* (CALB) in the kinetic resolution of the racemic chlorohydrin 4-(3-chloro-2 hydroxypropoxy)benzeneacetamide the enantiopure chlorohydrin was obtained with 99% *ee* [18]. We have now improved the yield of the building block and reduced the amounts of reactants used. The enantiopure drug (*S*)-atenolol has been synthesized from the enantiopure chlorohydrin.

2. Results and discussion

2.1. Deprotection of the phenol proton

The first step in the synthesis of (*S*)-atenolol ((*S*)-**4**) is the formation of 4-(3-chloro-2-hydroxypropoxy)benzeneacetamide (**2a**) and 2-(4-(2-oxiran-2-ylmethoxy)phenyl)benzeneacetamide (**2b**) from phenol 2-(4-hydroxyphenyl)acetamide (**1**), sodium hydroxide and epichlorohydrin. The impact of the amount of sodium hydroxide, epichlorohydrin and acetic acid used in the reaction was investigated, in order to improve the overall yield of the product compared to earlier reported data (Scheme 1).



Scheme 1. Synthesis of (*S*)-atenolol ((*S*)-**4**), starting from a sodium hydroxide catalyzed deprotonation of 2-(4-hydroxyphenyl)acetamide (**1**) with addition of epichlorohydrin giving a mixture of 4-(3-chloro-2-hydroxypropoxy)benzeneacetamide (**2a**) and 2-(4-(2-oxiran-2-ylmethoxy)phenyl)benzeneacetamide (**2b**). Subsequent addition of lithium chloride and acetic acid in tetrahydrofuran to the mixture gave pure **2a**, which was resolved in acetonitrile with vinyl butanoate and with lipase B from *Candida antarctica* (CALB). Addition of isopropylamine in water to (*R*)-**2a** (>99% *ee*) gave (*S*)-atenolol ((*S*)-**4**) in >99% *ee*, 60% yield and 99% purity.

2.2. Dimer Formation With Use of Stoichiometric Amount of Base

When stoichiometric amounts of sodium hydroxide was used to deprotonate the phenolic proton of **1** a small peak at t_R = 13.37 min is seen in the HPLC chromatogram together with the wanted

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product chlorohydrin **2a** (t_R = 12.06 min) and the epoxide **2b** (t_R = 9.58 min), see Figure 1. LC-MS analysis of the reaction mixture on an AQUITY UPLC BEH C18-column with isocratic mobile phase composition of water and acetonitrile and (30:70) with 1% formic acid and a flow of 0.2 mL/min showed a peak with the molecular mass of 382.04 g/mol, which corresponds to the mass of $C_{19}H_{21}N_2O_5Na$ (Figure 2). The compound has then a molecular mass of 358.39 g/mol, which corresponds to the dimer **2c**. We have previously predicted the mechanism of dimer formation in syntheses of similar beta-blockers [19].

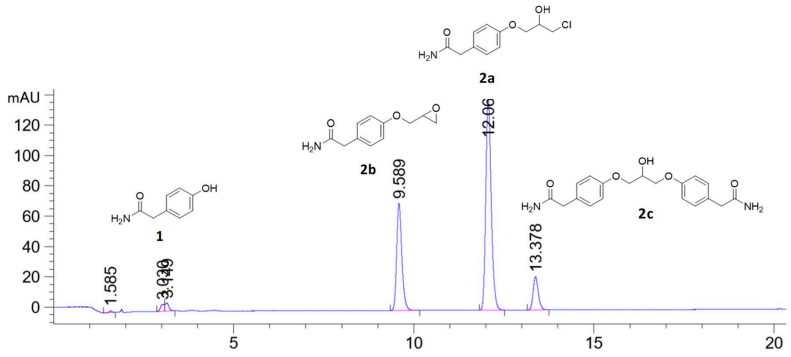

Figure 1. HPLC-chromatogram of the reaction starting with phenol **1** (t_R = 3.03 min), with stoichiometric amount of sodium hydroxide producing halohydrin **2a** (t_R = 12.06 min) and epoxide **2b** (t_R = 9.58 min) and the by-product dimer **2c** (t_R = 13.38 min) analyzed on an (achiral) Zorbax Eclipse XDB C18-column. A linear gradient mobile phase composition of water and acetonitrile (75:25) - (65:35) over 20 minutes with 0.5 mL/min flow was used.

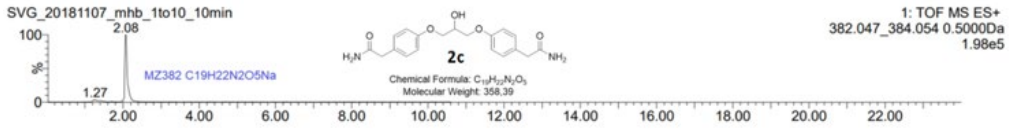

Figure 2. LC-MS analysis of the by-product **2c** showed a molecular mass of 382.04 g/mol, which corresponds to $C_{19}H_{21}N_2O_5Na$. The calculated mass of **2c** is 358.39 g/mol, $C_{19}H_{22}N_2O_5$, which is the evidence of this dimeric structure. The reaction mixture showed in the chromatogram in Figure 1 was analyzed on an AQUITY UPLC BEH C18-column with isocratic mobile phase composition of water and acetonitrile (30:70) with 1% formic acid and a flow of 0.2 mL/min.

2.3. Base Catalyzed Deprotection of Phenol 1

When two equivalents of epichlorohydrin were added to a solution containing phenol **1** and catalytical amounts of sodium hydroxide, the by-product **2c** was not observed after 4 hours of reaction. A proposed mechanism for the reaction of **1** to **2a** and **2b** with the regeneration of the base is shown in Scheme 2. Phenol **1** is deprotonated forming alkoxide **1'** which can attack epichlorohydrin at carbon 1 and 3 (reaction mechanism a and b, respectively) giving alkoxide **2a'** and epoxide **2b**. Alkoxide **2a'** reacts in two ways; by protonation from water forming chlorohydrin **2a,** thus regenerating the base (reaction mechanism c), and by an intramolecular S_N2-like reaction forming epoxide **2b** (reaction mechanism d).

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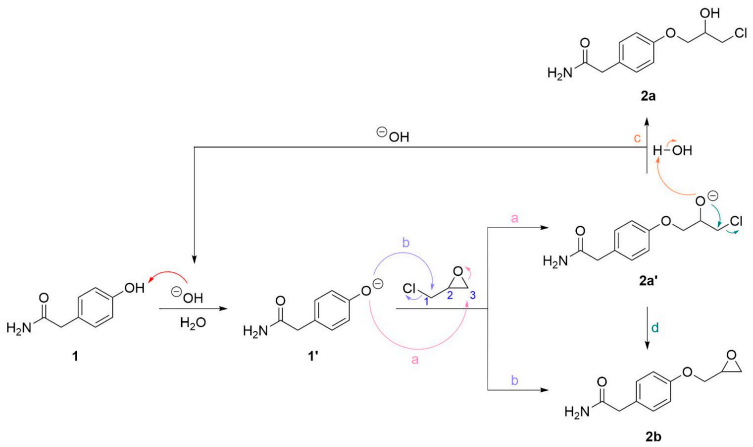

Scheme 2. Reaction mechanism for the sodium hydroxide catalyzed reaction of phenol **1** forming chlorohydrin **2a** and epoxide **2b**. Phenol **1** is deprotonated forming alkoxide **1'** which can attack epichlorohydrin at carbon 1 and 3 (reaction mechanism a and b, respectively) giving alkoxide **2a'** and epoxide **2b**. Alkoxide **2a'** reacts in two ways; by protonation from water forming chlorohydrin **2a,** thus regenerating the base (reaction mechanism c), and by an intramolecular S_N2-like reaction forming epoxide **2b** (reaction mechanism d).

Use of catalytic amounts of base is possible due to the regeneration of the base during the deprotonation of water during the formation of **2a** from **2a'**. For the same reason it is also possible to use less acetic acid than previously reported in the syntheses of other beta-blockers. We have previously used between 5 and 10 equivalents of acetic acid [20]. By the addition of one equivalent of lithium chloride and five equivalents of acetic acid to the mixture of **2a** and **2b**, a 52% yield of **2a** was achieved.

2.4. Lipase Catalyzed Kinetic Resolution of Chlorohydrin 2a

A CALB catalyzed kinetic resolution of **2a** with vinyl butanoate as the acyl donor produced (*R*)-**2a** in 99% *ee* with 32% yield (Scheme 1). Figur X shows the chromatogram of the reaction after 24 hours of a total of 27 hours reaction time. AS shown, the product ester S-2b was not separated on this column, however, it was hydrolyzed and analyzed as the S-2a as shown in outr previous published work on this topic. We have previously obtained a yield of 16% of (*R*)-**2a**, and the *E*-value of the kinetic resolution of **2a** was >200. In our previous article we also present data of the (*S*)-enantiomer of the chlorohydrin, (*S*)-**2a** [18].

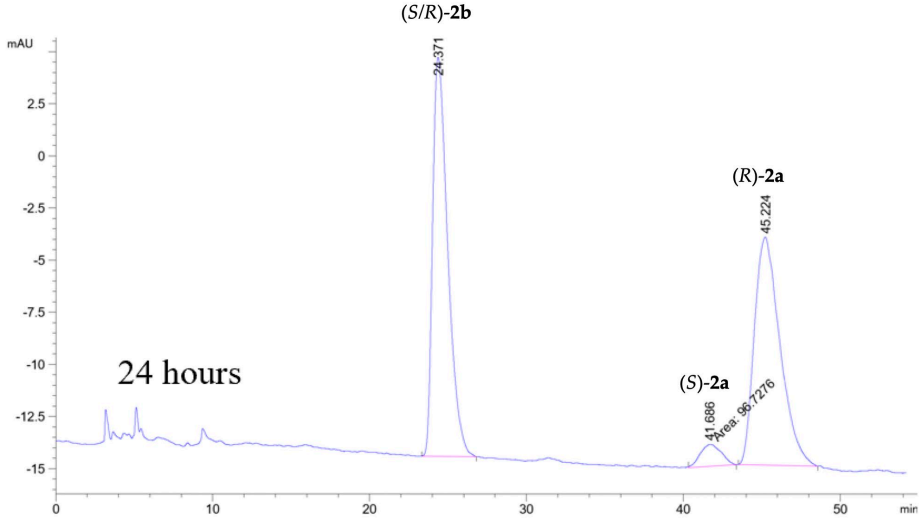

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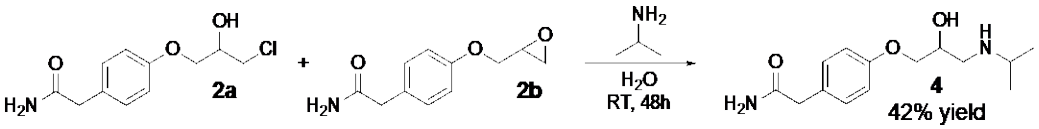
Figure 3. Kinetic resolution of **2a** catalyzed by CALB after 24 hours of reaction time. HPLC analyses were performed on a Chiralcel OD-H column (250 mm L x 4.6 mm i.d, 5 µm particle size) with isocratic eluent *i*-PrOH:*n*-hexane, 17:83, flow 1 mL/min. This gave unseparated ester enantiomers t_R (*R*/*S*)-**2b** = 25.37 min, t_R (*S*)-**2a** = 41.66 min and t_R (*R*)-**2a** = 45.22 min with R_S = 1.4. After 27 hours no (S)-2a was seen in ht echromatogram and the *ee* of (*R*)-**2a** was >99.0%.

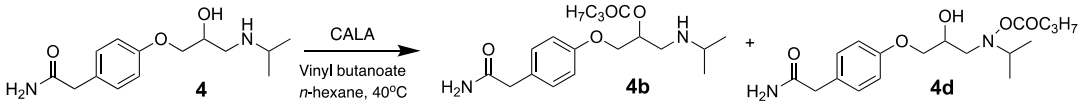
2.5. Synthesis of racemic atenolol (4) and attempt to resolve 4 with CALA

Racemic atenolol (**4**) was produced directly from the reaction mixture of **2a** and **2b** (without the ring opening of **2b** with lithium chloride) with addition of isopropylamine in water giving **4** in 42% yield (Scheme 3).



Scheme 3. Atenolol (**4**) was prepared directly from the reaction mixture of **2a** and **2b** with addition of isopropylamine in water giving **4** in 42% yield.

Attempts to resolve **4** in *n*-hexane with lipase A from *Candida antarctica* (CALA) as catalyst and vinyl butanoate as acyl donor was performed with an *E*-value of 1.8 (Scheme 4). We have previously had success with using CALA as catalyst in kinetic resolution of secondary alcohols with two (quite) large groups connected to the stereocenter, which atenolol also has [21].



Scheme 4. Kinetic resolution of **4** in dry *n*-hexane with lipase A from *Candida antarctica* (CALA) as the catalyst and vinyl butanoate as acyl donor at 40°C and 200 rpm showing low selectivity and several acetylation products.

The *ee* of the unreacted (*S*)-atenolol was 4% (ee_S ((*S*)-atenolol, (*S*)-**4**)) and *ee* for the product ester (*R*)-**4b** was 27% (ee_P). The retention times were assigned due to the known stereo preference of CALA [21], t_R (*S*)-**4** = 18.36 min and t_R (*R*)-**4** = 28.97 min, R_S = 2.72, t_R (*S*)-**4b** = 25.04 min and t_R (*R*)-**4b** = 33.87 min, Chiralcel OD-H column with a gradient mobile phase *i*-PrOH/*n*-hexane; 9:95 (0 min) -10:90 (10 min) - 60:40 (80 min), flow 0.5 mL/min (Figure 4).

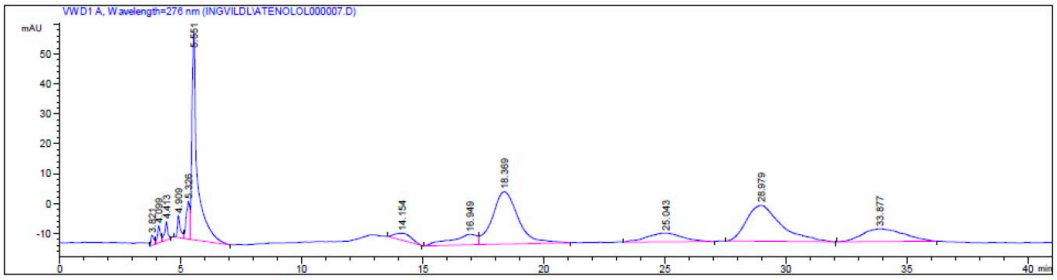


Figure 4. HPLC chromatogram of the kinetic resolution of atenolol (**4**) with CALA as the catalyst, showing retention times t_R (*S*)-**4** = 18.36 min and t_R-(*R*)-**4** = 28.97 min, the esters **4b** t_R (S)-**4b** = 25.04 min and t_R (*R*)-**4b** = 33.87 min and a by-product (t_R = 14.15 min and t_R = 16.94 min) analyzed on a Chiralcel OD-H column (250 mm L × 4.6 mm i.d., 5 µm particle size) with a gradient mobile phase *i*-PrOH/*n*-hexane; 9:95 (0 min) – 10:90 (10 min)- 60:40 (80 min), at 0.5 mL/min.

These results show that CALA exhibits low selectivity for the enantiomers of atenolol (**4**). The racemic compound with t_R = 14.15 min and t_R = 16.94 min is anticipated to be the amide product **4d**

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(Scheme 4). As mentioned, Chalupka (Sikora) *et al.* have succeeded in resolving racemic atenolol with *Candida rugosa* lipase with vinyl acetate as the acyl donor giving 94% *ee* of the (*S*)-atenolol acetate [11].

3. Materials and methods

3.1. Chemicals

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). *n*-Hexane was dried manually by adding molecular sieves (4Å) to the solvent 24 h before use. 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.

3.2. Enzymes

Candida antarctica Lipase B (CALB) (activity ≥ 10,000 PLU/g, 1 unit corresponds to the synthesis of 1 mmol per minute propyl laureate from lauric acid and 1-propanol at 60℃, lot no. 20170315), immobilized at high hydrophobic macroporous resin, produced in fermentation with genetically modified *Pichia pastoris* was gifted from Syncozymes Co, Ltd. (Shanghai, China). *Candida antarctica* lipase A (activity = 725 U/g, lot no. VZ1030-12, batch no 080116) immobilized on microporous beads was a gift from Viazym BV (Delft, The Netherlands).

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).

3.3. 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.

3.4. High-Performance Liquid Chromatography (HPLC)

Achiral HPLC analyses were performed on an Agilent 1290 system equipped with an auto injector (4 μL), detection was done by a diode array detector (DAD, l = 254 nm). All separations of **1, 2a**, and **2b** were performed on an Agilent Zorbax Eclipse XBD-C18 column (150 mm L × 4.6 mm i.d., 5 μm particle size) from Matriks (Oslo, Norway) with an isocratic eluent (H_2O:MeCN, 75:25) over 5 min with a flow of 1.0 mL/min, which gave t_R **1b** = 1.86 min, t_R **2b** = 3.04 min and t_R **2a** = 3.32 min. When stoichiometric amount of sodium hydroxide was used also the dimer **2c** was seen, then a linear gradient mobile phase composition of H_2O and MeCN (75:25) - (65:35) over 20 min with 0.5 mL/min flow was the method on the Zorbax Eclipse XDB C18-column, giving t_R **1** = 3.03 min, t_R **2a** = 12.06 min, t_R **2b** = 9.59 min and the by-product dimer t_R **2c** = 13.38 min.

LC-MS analysis of **2c** was performed on a ACQUITY UPLC BEH C18 column (100 mm L x 2.1 mm i.d., 1.7 μm particle size) from Waters™ (Waters Norway, Oslo, Norway) with isocratic mobile phase (H_2O:MeCN, 30:70) with 1% formic acid and a flow of 0.2 mL/min giving a mass of 382.04 g/mol which is which corresponds to $C_{19}H_{21}N_2O_5Na$. The calculated mass of **2c** is 358.39 g/mol, the formula for is $C_{19}H_{22}N_2O_5$.

Chiral HPLC analyses 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 L, x 4.6 mm i.d., 5 μm particle size) in addition to the reverse phase corresponding column chiralcel OD-RH (150 x 4.6 mm i.d., 5 μm particle size). The method used for all analyses was *i*-PrOH:*n*-hexane, 17:83, flow 1 mL min^{-1}, UV 254 nm. The enantiomers of **2a** eluted with t_R (*S*)-**2a** = 41.52 min, t_R (*R*)-**2a** = 46.32 min, R_S = 1.74. The purified (*R*)-**2a** was analyzed by the same method as racemic **2a**: t_R (*R*)-**2a** = 46.32 min, with no

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presence of (*S*)-**2a** in the chromatogram, resulting in an enantiomeric excess of > 99% *ee*. For determination of the *E*-value of the enzyme catalyzed kinetic reesolution of **2a**, see Lund *et al.* [18]. With the use of Chiralcel OD-RH (reverse phase) the retention times were t_R (*R*)-**2a** = 6.32 min and t_R (*S*)-**2a** = 7.17 min. Retention times of **4** on Chiralcel OD-H: t_R (*S*)-**4** = 18.37 min and t_R (*R*)-**4** = 28.98 min, R_S = 2.72. Purified (*S*)-atenolol: t_R (*S*)-**4** = 18.39 min.

3.5. 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 (Bruker, Rheinstetten, Germany). Chemical shifts are in ppm relative to TMS and coupling constants are in hertz (Hz).

3.6. Mass Spectroscopy Analyses

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).

3.7. Infrared Spectroscopy Analyses

Infrared spectroscopy was performed on a NEXUS FT-IR model 470 instrument from Thermo Nicolet Corporation (Madison, WI, USA).

3.8. Specific Rotation Analyses

Specific rotation was determined on a PerkinElmer Model 341 Polarimeter (Waltham, MA, USA), with a cell of 10 cm length, λ 589 nm.

3.9. Assignment of Absolute Configurations

Absolute configuration of the faster reacting enantiomer in lipase catalyzed resolution was determined by the known enantioselectivity of CALA [21] and CALB [22] and by comparing the elution orders of the atenolol building block enantiomers and drug enantiomer with previous analyses of similar α-halogenated 1-(4-benzyloxy)phenyl)ethanols on the Daicel Chiralcel OD-H column. In general, the *R*-enantiomers are the most retained [23].

3.10. Synthesis Protocols

3.10.1. Synthesis of chlorohydrin 2a and epoxide 2b

To an aqueous solution of NaOH (0.2M, 8.71 mL, 1.98 mmol) and 2-(4-hydroxyphenyl)-acetamide (**1**) (1.00 g, 6.62 mmol), epichlorohydrin (1.04 mL, 13.26 mmol) were added and set to stir at rt. for 4 h. The reaction was monitored by TLC (MeOH:CH_2Cl_2, 1:5): R_f **2a** = 0.56, R_f **2b** = 0.63. The reaction mixture was washed with H_2O *in vacuo* giving a 1.44 g mixture of chlorohydrin **2a** and epoxide **2b** as a white solid, analyzed on the Zorbax Eclipse XBD-C18 HPLC column with an isocratic mobile phase (MeCN:H_2O:, 25:75) over 5 min, flow = 1.0 mL/min, t_R (**1**) = 1.86 min, t_R **2a** =3.32 min and t_R **2b** = 3.04 min.

3.10.2. Synthesis of chlorohydrin 2a by ring-opening of epoxide 2b

To a mixture of chlorohydrin **2a** and epoxide **2b** (1.44 g), MeCN (20 mL), LiCl (0.28 g, 6.6 mmol) and AcOH (1.98 mL, 34.62 mmol) was added and stirred at rt. for 24 h. The reaction was monitored by TLC (CH_2Cl_2:MeOH, 1:5), R_f **2a** = 0.56. The reaction mixture was quenched with Na_2CO_3 (pH 12) to a neutral pH followed by extraction with CH_2Cl_2 (3 x 50 mL). The organic layer was dried over

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$MgSO_4$ and the solvent was removed under reduced pressure and further *in vacuo* giving chlorohydrin **2a** in 52% yield (0.85 g, 3.49 mmol) as a white solid; mp. 143-145°C. The conversion of epoxide **2b** was analyzed on the Zorbax Eclipse XBD-C18 HPLC column with an isocratic mobile phase (MeCN:H_2O:, 25:75) over 5 min, flow = 1.0 mL/min, t_R **2a** =3.32 min. ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm: 7.38 (s, 1H, -NH-H), 7.17-7.16 (m, 2H aromatic), 6.88-6.86 (m, 2H, aromatic), 6.82 (s, 1H, -NH-H), 5.54-5.53 (d, 1H, $^3J_{HH}$ = 5.13 Hz, -OH), 4.04-4.00 (sextet, 1H, $^3J_{HH}$ = 5.13 Hz, -CH-OH), 3.97-3.92 (m, 2H, -O-CH_2-), 3.68-3.65 (2H, m, 2H, CH_2-Cl), 3.28 (s, 2H, -CH_2-$CONH_2$); ^{13}C NMR (600 MHz, DMSO-d_6) δ ppm: 172.9, 157.5, 130.5 (2C), 129.2, 114.7 (2C), 69.5, 69.1, 47.2, 41.8. MS (TOF-ASAP): $[M+H]^+$ 244.0739, (calcd. $C_{11}H_{14}NO_3Cl$, 243.654). IR (cm^{-1}): 3349, 1633, 1241, 706.

3.10.3. Synthesis of Racemic Atenolol (4) Directly from Phenol 1

2-(4-Hydroxyphenyl)acetamide (**1**) (2.52 g, 16.67 mmol) was stirred in 2-(chloromethyl)oxirane (epichlorohydrin) (13 mL, 165.80 mmol) at rt, and was added a solution of NaOH (0.33 g, 8.25 mmol) in H_2O (5 mL). After 48 h, full conversion was observed by TLC (MeOH: CH_2Cl_2: 1:4), which showed the presence of both **2a** and **2b**. The mixture was filtered, and the solids were dried under reduced pressure, before the crude product was dissolved in MeOH (25 mL) and added *i*-$PrNH_2$ (10 mL, 116.39 mmol). After stirring for 24 h, full conversion of **1** to **4** was observed by TLC. The solvent was removed under reduced pressure. This afforded 5.49 g crude product of which 1.03 g was recrystallized from MeCN, afforded atenolol (**4**) in 42% total yield (0.35 g, 1.31 mmol) and > 98% purity (NMR). ^{1}H NMR (400 MHz, CD_3OD) δ ppm: 7.26-7.24 (d, 2H, aromatic, $^3J_{HH}$ = 7.5 Hz), 6.95-6.93 (d, 2H, aromatic, $^3J_{HH}$ = 7.5 Hz), 4.24-4.22 (m, 1H, CH-OH, $^3J_{HH}$ = 4.4 Hz), 4.08-3.99 (m, 2H, -O-CH_2-, $^3J_{HH}$ = 5.0 Hz, 9.5 Hz), 3.49-3.43 (m, 3H, CO-CH_2-, -NH-CH-, $^3J_{HH}$ = 6.6 Hz), 3.30-3.27 (m, 1H, -CH_2-NH-, $^3J_{HH}$ = 13.2 Hz), 3.18-3.12 (t, 1H, -CH_2-NH-, $^3J_{HH}$ = 10.5 Hz), 1.39-1.38 (m, 6H, $^3J_{HH}$ = 3.5 Hz). ^{13}C NMR (400 MHz, CD_3OD) δ ppm: 175.8, 157.5, 129.9, 128.4, 114.3, 69.6, 65.6, 50.6, 47.1, 41.0, 18.0, 17.4.

3.10.4. Synthesis of (R)-2-(4-(3-chloro-2 hydroxypropoxy)phenyl)acetamide, (R)-2a

Chlorohydrin **2a** (0.56 g, 2.3 mmol) and vinyl butanoate (1.43 g, 12.5 mmol) were added to a flask with dry MeCN (40 mL) and molecular sieve. CALB (0.71 g) was added, and the reaction was incubated at 30°C and 200 rpm for 27 h in an incubator shaker. The enzyme and the molecular sieves were filtered off and the solvent was removed under reduced pressure. The ester (*S*)-**3** and the chlorohydrin (*R*)-**2a** were separated on a silica column with EtOAc as eluent. This yielded (*R*)-**2a** (0.090 g, 0.37 mmol, 32% yield), *ee* > 99%. $[\alpha]_D^{23}$= −3.0 (1.0, MeOH), which is in accordance with our previous reported data of (*R*)-**2a** [18]. The *E*-values and K_{eq} were calculated by the software program *E&K Calculator 2.1b0 PPC* [24].

3.10.5. Synthesis of (S)-Atenolol, (S)-4

To (*R*)-**2a** (0.090 g, 0.37 mmol) *i*-$PrNH_2$ (3 mL, 34.9 mmol), and dist. H_2O (1.0 mL) was added. The reaction was stirred at room temp. for 48 h until TLC (MeOH:CH_2Cl_2, 1:5) showed full conversion. This gave (*S*)-**4** as a white powder (0.054 g, 0.22 mmol, 60% yield), 99% purity (NMR), *ee* > 99%. $[\alpha]_D^{23}$= −17.0 (1.0, 1N HCl). NMR spectra as for racemic **4**.

3.10.6. Kinetic resolution of 2-(4-(2-hydroxy-3 (isopropylamino)propoxy)phenyl)acetamide (4)

To a solution of 2-(4-(2-hydroxy-3-(isopropylamino)propoxy)phenyl)acetamide (**4**) (35.1 mg, 0.13 mmol) and vinyl butanoate (81.3 mg, 0.71 mmol) in MeCN (3 mL) placed in an incubator shaker at 30°C and 200 rpm, CALA (20.4 mg) was added. Samples were collected over four days, which were analyzed by chiral HPLC (*n*-hexane:*i*-$PrNH_2$:Et_2NH: 80:20:0.1) which showed low enantioselectivity (*E* = 1.8) and the reaction was therefore not further analyzed.

4. Conclusions

A four-step synthesis of (*S*)-atenolol ((*S*)-**4**) in >99% *ee* has been performed, starting from 2-(4-hydroxyphenyl)-acetamide (**1**). Base catalyzed deprotonation of the starting material avoided

formation of the dimer by-product (**2c**), thus increasing the overall yield of the racemic chlorohydrin **2a** to 52%, an improvement from our previous reported yield of 22%. CALB catalyzed kinetic resolution of chlorohydrin **2a** gave the enantiopure (*R*)-**2a** in >99% *ee* with 32% yield. CALB catalyzed kinetic resolution of chlorohydrin **2a** is an efficient method to obtain enantiopure building blocks for beta-blockers, however, the yield of this enzymatic step is limited to 50%. By use of f. inst. dynamic kinetic resolution inverting the "wrong" enantiomer to the right one, the yield could be increased further [25]. Our new protocol to achieve enantiopure (*S*)-atenolol has fewer steps and uses reduced amounts of all reagents in the synthesis compared to previous published protocols.

Supplementary Materials: The following supporting information can be downloaded at: Preprints.org, Chromatograms.

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