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# Chemo-Enzymatic Synthesis of Enantiopure (−)-Nebivolol Catalyzed by Lipase B from *Candida antarctica*

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**Abstract**

All four isomers of 2-chloro-1-(6-fluorochroman-2-yl)ethan-1-ol, as building blocks for the two enantiomers of beta-blocker nebivolol, have been synthesized in high yield. Due to the similar physicochemical properties of these four diastereomeric halohydrins, to date, the only successful method for separation of the isomers has been preparative HPLC. To avoid this, the four halohydrins were transformed into epoxides with subsequent separation of the enantiomeric pairs by column chromatography. The enantiomeric pairs of epoxides were subsequently converted back to their corresponding halohydrins before performing kinetic resolution of the racemates catalyzed by Lipase B from *Candida antarctica*. (*R*)-2-Chloro-1-((*R*)-6-fluorochroman-2-yl)ethanol was isolated in 71% yield, and >99% enantiomeric excess (*ee*). (*R*)-2-Chloro-1-((*S*)-6-fluorochroman-2-yl)ethanol was isolated in 77% yield and >99% *ee*. Hydrolysis of 2-chloro-1-(6-fluorochroman-2-yl)ethyl butanoate with the same lipase yielded halohydrins (*S*)-2-chloro-1-((*S*)-6-fluorochroman-2-yl)ethanol and (*S*)-2-chloro-1-((*R*)-6-fluorochroman-2-yl)ethanol. Amination of (*R*)-6-fluoro-2-((*S*)-oxiran-2-yl)chromane with ammonia afforded (*S*)-2-amino-1-((*R*)-6-fluorochroman-2-yl)ethanol in 79% yield and >99% *ee*. (*S*)-2-Amino-1-((*R*)-6-fluorochroman-2-yl)ethanol was then reacted with (*R*)-2-chloro-1-((*S*)-6-fluorochroman-2-yl)ethanol to produce the desired product (*R*,*S*,*S*,*S*)-nebivolol ((−)-nebivolol) in 81% yield and >99% *ee*.

**Keywords:** (−)-nebivolol; enantiopure building blocks; *Candida antarctica* lipase B

## 1. Introduction

Beta-blocker nebivolol consists of a racemic mixture of enantiomers (*R*,*S*,*S*,*S*)-nebivolol ((−)-nebivolol or l-nebivolol) and (*S*,*R*,*R*,*R*)-nebivolol ((+)-nebivolol or d-nebivolol) [1] (Figure 1).

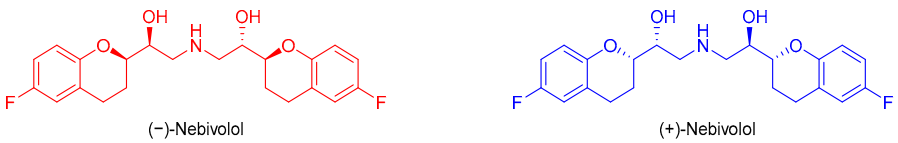



**Figure 1.** (*R*,*S*,*S*,*S*)-2,2′-azanediylbis(1-(6-fluorochroman-2-yl)ethanol ((−)-nebivolol), shown in red on the left, and (*S*,*R*,*R*,*R*)-2,2′-azanediylbis(1-(6-fluorochroman-2-yl)ethanol ((+)-nebivolol), shown in blue on the right.

2,2′-Azanediylbis(6-fluorochroman-2-yl)ethan-1-ol was developed and patented as a pharmaceutical drug called nebivolol in the 1980s [1,2]. Nebivolol entered the European

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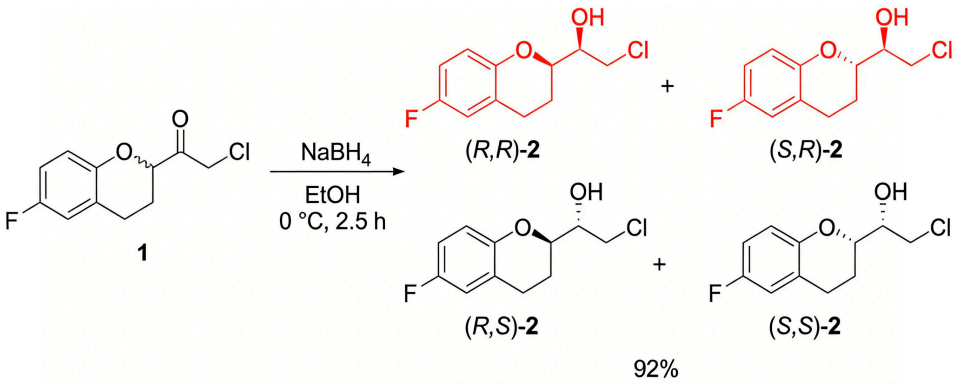
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market in 1997 as Lobivon®. The drug received FDA approval in the United States in 2007 under trade names Nebilet® and Bystolic®. Both pharmaceuticals are marketed with enantiomers (*R*,*S*,*S*,*S*)- and (*S*,*R*,*R*,*R*)-nebivolol in the form of their hydrochloride salts as the Active Pharmaceutical Ingredient (API) [2,3]. The (+)-enantiomer of nebivolol functions as a highly selective beta$_1$-adrenergic receptor antagonist in the heart, effectively inhibiting the binding of adrenaline [4], and is therefore primarily responsible for the cardiac effects of the drug [5]. The remarkably high eudismic ratio of (+)-nebivolol of 175 underscores its potency, making it 175 times more effective as a beta-blocker than (−)-nebivolol [3,4]. In contrast, (−)-nebivolol predominantly stimulates beta-adrenergic receptors, leading to a release of nitric oxide (NO) from endothelial cells [6]. This promotes vasodilation, relaxation of vascular smooth muscle, and a consequent reduction in blood pressure [5]. Notably, (−)-nebivolol demonstrates greater vasodilatory activity in human endothelial cells than both the racemic mixture and enantiopure (+)-nebivolol by enhancing nitric oxide (NO) production, in addition to possessing strong antioxidant properties, reducing superoxide radicals, which improves cellular NO metabolism and activates β3-adrenoceptors. This action, along with its antioxidants effects, contributes to nebivolol's vasodilatory effects [7]. 2,2′-Azanediylbis(1-(6-fluorochroman-2-yl)ethanol) contains four stereocenters, which when synthesized, result in 16 possible stereoisomers. However, due to the presence of a symmetry plane that passes through the nitrogen atom of the molecule, the total number of unique isomeric structures is reduced to 10. In 2006, Tuchalski et al. successfully isolated nine of these isomers using preparative HPLC and characterized them by X-ray spectroscopy, giving the absolute configuration of all nine isomers. Specific optical rotation values of the isomers were reported [8]. According to FDA guidelines, drugs with stereogenic centers should be developed as single enantiomers when the enantiomers of a racemate differ in activity, since the less active enantiomer may act as an impurity and cause adverse effects [9]. To address this, we previously reported our protocol for (*S*,*S*,*S*,*R*)-nebivolol in 100% *ee* catalyzed by a ketoreductase from Syncozymes Co., LTD, Shanghai, China [10]. Here, we present the synthesis of (*R*,*S*,*S*,*S*)-nebivolol with *Candida antarctica* lipase B as a catalyst in kinetic resolutions of the chlorohydrin building blocks, achieving >99% enantiomeric excess of the two chiral building blocks and the (*R*,*S*,*S*,*S*)-enantiomer. We have previously had success with producing single enantiomers of chiral drugs with the same enzyme catalysts [11–19]. Recently, Xue et al. (2024) published a study reporting on alcohol dehydrogenases from several microorganisms as catalysts for the nebivolol building blocks [20]. It is not clear if these enzymes are commercially available.

## 2. Results and Discussion

### *2.1. Synthesis of Halohydrins* **2** *from the Corresponding Ketone* **1**

2-Chloro-1-(6-fluorochroman-2-yl)ethanone (**1**), the starting material for the chiral building blocks of (−)-nebivolol, was synthesized in 80% yield from commercially available 6-fluorochromane-2-carboxylic acid following the procedure reported in our previous publication by Bjørnes et al. [10]. The four isomers of **2** were synthesized from chloroketone (**1**) in ethanol (97%) at 0 °C for 2.5 h, using sodium borohydride as the reducing agent, with a yield of 92%, which is slightly better than we previously reported (Scheme 1).




**Scheme 1.** Synthesis of **2** in 92% yield by reduction of chloroketone **1** with sodium borohydride. (*R*,*R*)-**2** and (*S*,*R*)-**2** are the building blocks for (−)-nebivolol (both in red).

HPLC analysis of halohydrins **2** was performed on a Chiralpak AD-H column with *n*-hexane and 2-propanol (90:10, *v*/*v*) as the mobile phase and a flow rate of 1 mL/min. The retention times were $t_R$((*S*,*R*)-**2**) = 8.43 min, $t_R$((*R*,*S*)-**2**) = 9.21 min, $t_R$((*S*,*S*)-**2**) = 10.96 min and $t_R$((*R*,*R*)-**2**) = 11.65 min (Figure 2). The absolute configuration notation starts from left to right in the molecules, in accordance with most literature about nebivolol and its chiral building blocks.

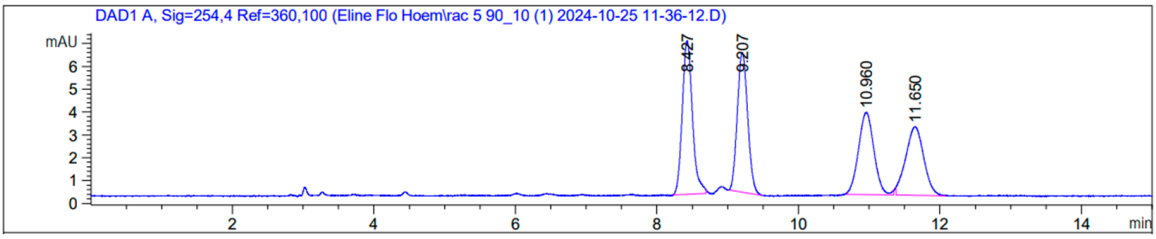
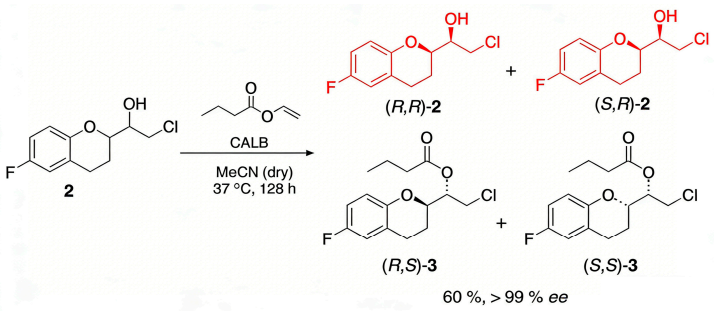



**Figure 2.** HPLC chromatogram of racemic halohydrins **2** obtained on a Chiralpak AD-H column with *n*-hexane and 2-propanol (90:10, *v*/*v*) as the mobile phase and a flow rate of 1 mL/min. The retention times are $t_R$((*S*,*R*)-**2**) = 8.43 min., $t_R$((*R*,*S*)-**2**) = 9.21 min., $t_R$((*S*,*S*)-**2**) = 10.96 min. and $t_R$((*R*,*R*)-**2**) = 11.65 min. All peaks are baseline-separated; $R_S$ > 1.5.

### *2.2. CALB Catalyzed Transesterification of* **2**

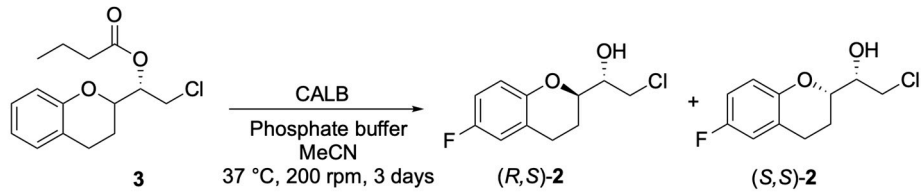
In order to separate (*R*,*R*)-**2** and (*S*,*R*)-**2** from the reaction mixture of **2**, lipase B from *Candida antarctica* (CALB) from Syncozymes Co. LTD (Shanghai, China) was used to catalyze a kinetic resolution of **2** in dry acetonitrile with vinyl butanoate as acyl donor, giving butanoate esters (*S*,*S*)-**3** and (*R*,*S*)-**3** (Scheme 2). The esters were then separated from the remaining alcohols, i.e., (*R*,*R*)-**2** and (*S*,*R*)-**2,** by column chromatography. Absolute configuration of the product esters and the remaining alcohols was based on comparison of the specific rotation value of the final product, (*S*,*R*,*R*,*R*)-**6**, with the specific rotation value reported by Tuchalski et al. [8]. The absolute configuration of the remaining alcohols and the ester products is also confirmed by our previous results using CALB in transesterifications of similar secondary alcohols [11–18].




**Scheme 2.** Transesterification of **2** catalyzed by Lipase B from *Candida antarctica* (CALB) in dry acetonitrile with vinyl butanoate as acyl donor, giving the corresponding esters, i.e., (*R*,*S*)-**3** and (*S*,*S*)-**3** from (*R*,*S*)-**2** and (*S*,*S*)-**2**, respectively.

### *2.3. CALB Catalyzed Hydrolysis of (R,S)-***3** *and (S,S)-***3**

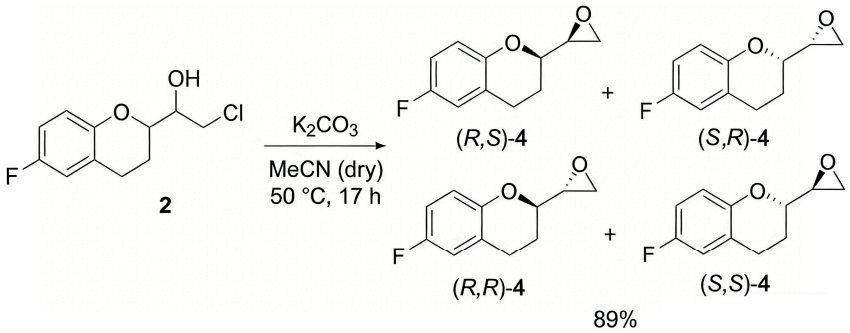
By hydrolysis of the mixture of the (*R*,*S*)-**3** and (*S*,*S*)-**3** esters by CALB, we obtained a mixture of the chiral building blocks for (+)-nebivolol ((*R*,*S*)-**2** and (*S*,*S*)-**2**) in 40% yield (see Scheme 3). This means that CALB catalysis of the halohydrins **2** can give both enantiomers of nebivolol (for further synthesis steps of (+)-nebivolol from (*R*,*S*)-**2** and (*S*,*S*)-**2**, see Bjørnes et al. (2025) [10]).




**Scheme 3.** CALB-catalyzed hydrolysis of the mixture of esters (*R*,*S*)-**3** and (*S*,*S*)-**3** gave a mixture of (*R*,*S*)-**2** and (*S*,*S*)-**2** in 40% yield. These two isomers of **2** are the chiral building blocks for (+)-nebivolol [10].

### *2.4. Converting* **2** *into Epoxides* **4**

In order to avoid tedious evaporation tasks after separation of the isomers of **2** on preparative HPLC, as reported in Bjørnes et al. [10], the four isomers of **2** were converted into the corresponding epoxides **4** with potassium carbonate in dry acetonitrile at 50 °C for 17 h (Scheme 4). The protocol is based on that reported by Zhang et al. [20].




**Scheme 4.** The four isomers of epoxide **4** were obtained in 89% yield from the reaction of **2** under basic conditions in dry acetonitrile.

The four isomers of **4** were obtained in equal amounts in 89% yield and >99% purity: (*R*)-6-fluoro-2-((*S*)-oxiran-2-yl)chromane ((*R*,*S*)-**4**), (*S*)-6-fluoro-2-((*R*)-oxiran-2-yl)chromane ((*S*,*R*)-**4**), (*S*)-6-fluoro-2-((*S*)-oxiran-2-yl)chromane ((*S*,*S*)-**4**) and (*R*)-6-fluoro-2-((*R*)-oxiran-2-yl)chromane ((*R*,*R*)-**4**). HPLC analysis of **4** was performed on a Chiralpak AD-H column

with *n*-hexane and 2-propanol (85:15, *v*/*v*) as the mobile phase and a flow rate of 1 mL/min. The retention times were $t_R$((*S*,*S*)-**4**) = 4.58 min., $t_R$((*R*,*R*)-**4**) = 4.74 min., $t_R$((*S*,*R*)-**4**) = 5.17 min. and $t_R$((*R*,*S*)-**4**) = 5.44 min. The latter peaks are baseline-separated; RS > 1.5 (Figure 3). The chromatogram peaks are of different heights, but the areas under the peaks are equal.

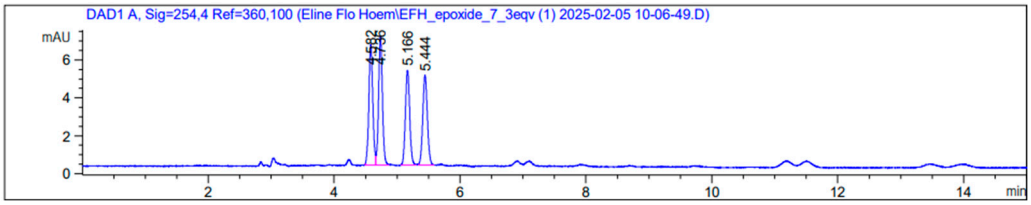
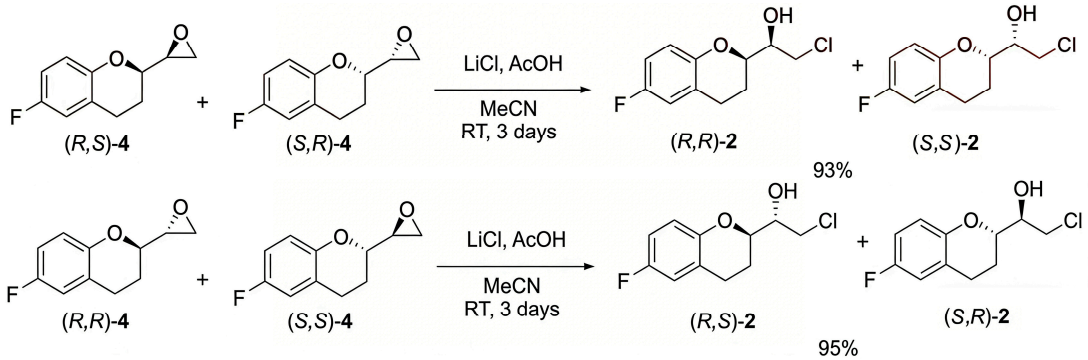



**Figure 3.** HPLC chromatogram of the isomers of epoxide **4** obtained on a Chiralpak AD-H column with *n*-hexane and 2-propanol (85:15, *v*/*v*) as the mobile phase and a flow rate of 1 mL/min., with retention times of $t_R$((*S*,*S*)-**4**) = 4.58 min, $t_R$((*R*,*R*)-**4**) = 4.74 min., $t_R$((*S*,*R*)-**4**) = 5.17 min. and $t_R$((*R*,*S*)-**4**) = 5.44 min. The chromatogram peaks are of different heights, but the areas under the peaks are equal.

The enantiomeric pairs of epoxide **4** were then separated by gravity column chromatography with *n*-hexane:EtOAc in a ratio of 100:0-40:60, giving (*R*,*R*)- and (*S*,*S*)-**4** in one fraction and (*S*,*R*)- and (*R*,*S*)-**4** in another fraction. The separation of the two fractions confirmed that the compound exists as two pairs of enantiomers. The elution pattern supports the fact that enantiomers cannot be separated on an achiral column but diastereomers can be: (*S*,*R*)-**4** and (*R*,*S*)-**4** eluted together in one fraction, while (*S*,*S*)-**4** and (*R*,*R*)-**4** eluted in another fraction.

To regenerate the enantiomeric pairs of the halohydrins **2** from the epoxides **4** after separation of the enantiomeric pairs, a ring-opening reaction of the epoxides was performed using lithium chloride and acetic acid in acetonitrile at room temperature for 3 days (Scheme 5). This yielded enantiomers (*R*,*R*)-**2** and (*S*,*S*)-**2** in 93% yield and >99% purity and enantiomers (*R*,*S*)-**2** and (*S*,*R*)-**2** in 95% yield and >99% purity. Since the absolute configurations of the halohydrins **2** had already been determined, the elution order of epoxides **4** could be assigned accordingly.

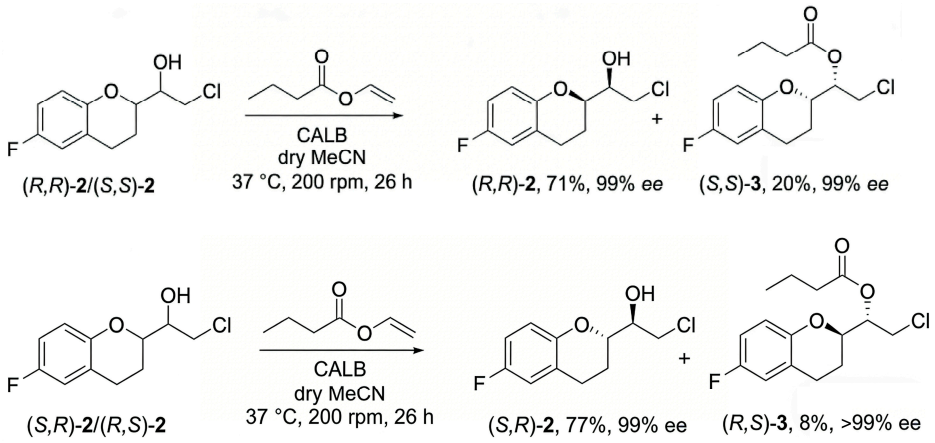



**Scheme 5.** Synthesis of halohydrins **2** by ring-opening reactions of the separated fractions of epoxides **4** with lithium chloride and acetic acid in acetonitrile at room temperature. Upper panel: (*R*,*R*)-**2** and (*S*,*S*)-**2** were obtained in 93% yield. Lower panel: (*R*,*S*)-**2** and (*S*,*R*)-**2** were obtained in 95% yield.

### *2.5. Kinetic Resolution of* **2** *Catalyzed by Lipase B from Candida antarctica and Obtention of Epoxide (R,S)-***4**

When the two enantiomeric pairs of **2** were separated as epoxide intermediates **4** and reacted back to the halohydrins **2**, the kinetic resolutions of the pairs—(*R*,*R*)-**2** and (*S*,*S*)-**2** and (*R*,*S*)-**2** and (*S*,*R*)-**2**—were catalyzed by CALB in separate reactions using vinyl

butanoate as the acyl donor (Scheme 6). The reactions were performed in anhydrous acetonitrile and incubated at 37 °C and 200 rpm for 26 h. Halohydrins **2** and the corresponding esters **3** were separated by gravity column chromatography (*n*-hexane:EtOAc, 100:0-40:60). HPLC analysis revealed that (*R*,*R*)-**2** was obtained in 71% yield and >99% *ee*. (*S*,*S*)-**3** was obtained in 20% yield and >99% *ee*. (*S*,*R*)-**2** was obtained in 77% yield and >99% *ee*. (*R*,*S*)-**3** was obtained in 8% yield and >99% *ee*.




**Scheme 6.** Enzymatic enrichment of the enantiomeric excess of the two enantiomeric pairs of halohydrins (**2**) by transesterification catalyzed by CALB in acetonitrile and with vinyl butanoate as acyl donor.

The esters and halohydrins shown in Scheme 6 were separated by column chromatography, and the ester from each reaction was hydrolyzed as described in Scheme 3 and in Section 3.5.11., giving (*R*,*S*)-**2** in 33% yield (Section 3.5.12). The yield of (*S*,*S*)-**2** from the hydrolysis of (*S*,*S*)-**3** was 31% (Section 3.5.13). The product, diastereomers (*R*,*S*)-**2** and (*S*,*S*)-**2**, from the ester hydrolysis of **3** are the building blocks for (+)-nebivolol. We are in the process of improving these hydrolysis reactions in order to be able to measure the specific rotation of halohydrins (*R*,*S*)-**2** and (*S*,*S*)-**2**.

HPLC analysis of the product ester and remaining halohydrin was conducted after the kinetic resolution of (*R*,*R*)-**2** and (*S*,*S*)-**2** was determined on a Chiralpak AD-H column with *n*-hexane and 2-propanol (85:15, *v*/*v*) as the mobile phase and a flow rate of 1 mL/min (Figures 4 and 5).

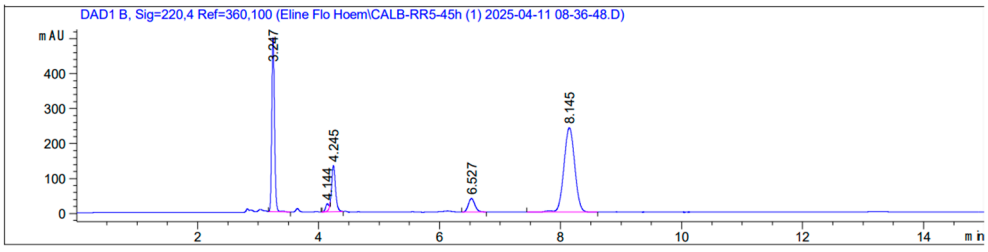



**Figure 4.** HPLC chromatogram of CALB-catalyzed kinetic resolution of (*R*,*R*)-**2** and (*S*,*S*)-**2** (before conversion of the halohydrins to epoxides) analyzed on a Chiralpak AD-H column with *n*-hexane and 2-propanol (85:15, *v*/*v*) as the mobile phase and a flow rate of 1 mL/min. The retention times were $t_R$((*R*,*S*)-**3**) = 4.14 min, $t_R$((*S*,*S*)-**3**) = 4.25 min, $t_R$((*S*,*R*)-**2**) = 6.53 min and $t_R$((*R*,*R*)-**2**) = 8.15 min. The peak at 3.25 min. is excess vinyl butanoate, which was later removed.

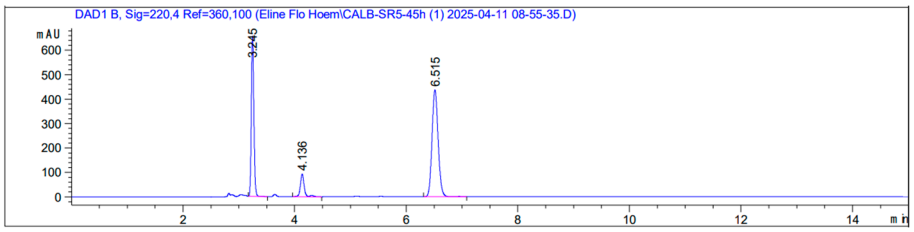



**Figure 5.** HPLC chromatogram of the CALB-catalyzed kinetic resolution of (*R*,*S*)-**2** and (*S*,*R*)-**2** analyzed on a Chiralpak AD-H column with *n*-hexane and 2-propanol (85:15, *v*/*v*) as the mobile phase and a flow rate of 1 mL/min. Retention times $t_R$((*R*,*S*)-**3**) = 4.14 min and $t_R$((*S*,*R*)-**2**) = 6.52 min. All of the alcohol (*R*,*S*)-**2** was esterified to butanoate (*R*,*S*)-**3**. The peak at 3.25 min is excess vinyl butanoate, which was later removed.

(*R*,*R*)-**2** was obtained in 82% diastereomeric excess (*de*). Some fractions were evaluated using only TLC, which may have resulted in missed detection of minor impurities. To address this, the mixture of (*R*,*R*)-**2** and (*S*,*R*)-**2** was converted to the corresponding epoxides, i.e., (*R*,*S*)-**4** and (*S*,*S*)-**4**, which were separated again by column chromatography. This solution gave (*R*,*S*)-**4** in 69% yield and >99% *ee*. (*S*,*S*)-**4** was not characterized further by NMR, since this isomer was not used as building block in the final step of (−)-nebivolol. Ester (*S*,*S*)-**3** with 80% *de* was separated from (*R*,*S*)-**3** by gravity column chromatography (*n*-hexane:EtOAc, 9:1).

HPLC analyses of halohydrins (*S*,*R*)-**2** and (*R*,*R*)-**2** (Figures 6 and 7) and epoxides (*R*,*S*)-**4** and (*S*,*S*)-**4** (Figures 8 and 9) were performed on a Chiralpak AD-H column.

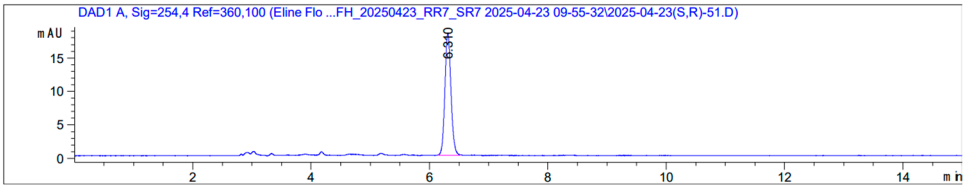



**Figure 6.** HPLC chromatogram of (*S*,*R*)-**2** analyzed on a Chiralpak AD-H column with *n*-hexane and 2-propanol (85:15, *v*/*v*) as the mobile phase, a flow rate of 1 mL/min, and a retention time of $t_R$((*S*,*R*)-**2**) = 6.31 min.

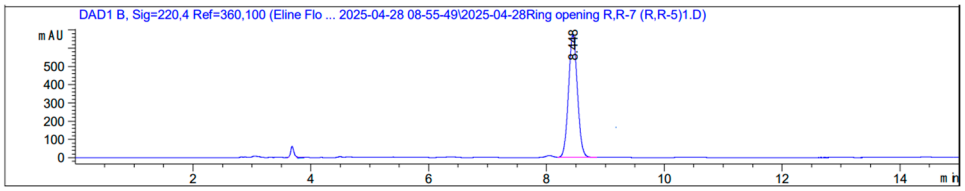



**Figure 7.** HPLC chromatogram of (*R*,*R*)-**2** analyzed on a Chiralpak AD-H column with *n*-hexane and 2-propanol (85:15, *v*/*v*) as the mobile phase, a flow rate of 1 mL/min, and a retention time of $t_R$((*R*,*R*)-**2**) = 8.45 min.

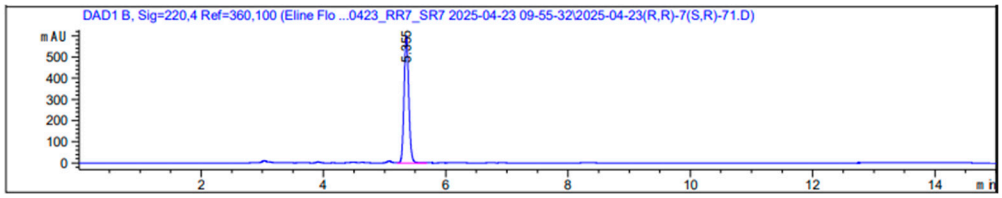



**Figure 8.** HPLC chromatogram of epoxide (*R*,*S*)-**4** obtained on a Chiralpak AD-H column with *n*-hexane and 2-propanol (85:15, *v*/*v*) as the mobile phase, a flow rate of 1 mL/min, and a retention time of $t_R$((*R*,*S*)-**4**) = 5.36 min.

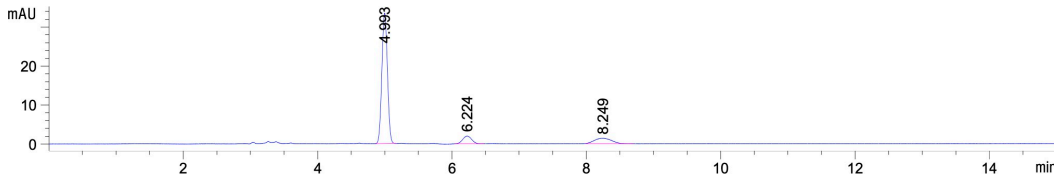
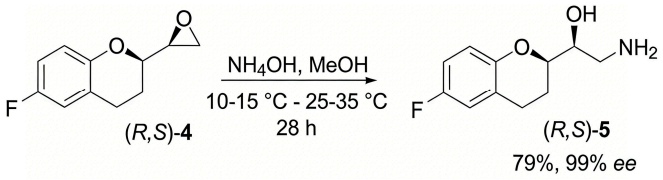



**Figure 9.** HPLC chromatogram of epoxide (*S*,*S*)-**4** obtained on a Chiralpak AD-H column with *n*-hexane and 2-propanol (85:15, *v*/*v*) as the mobile phase, a flow rate of 1 mL/min, and a retention time of $t_R$((*S*,*S*)-**4**) = 4.99 min.

### 2.6. Amination of (*R*,*S*)-**4**

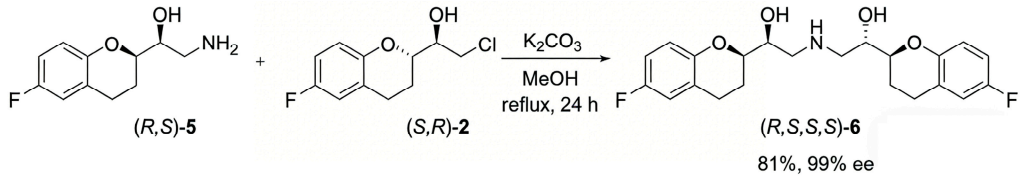
Enantiopure epoxide (*R*,*S*)-**4** was reacted with ammonia (25%) in methanol, giving (*S*)-2-amino-1-((*R*)-6-fluorochroman-2-yl)ethanol ((*R*,*S*)-**5**) in 79% yield and >99% *ee* (Scheme 7). A modified version of the reaction protocol described by Haldar et al. [3] was used.




**Scheme 7.** Synthesis of (*S*)-2-amino-1-((*R*)-6-fluorochroman-2-yl)ethanol ((*R*,*S*)-**5**) in 79% yield and >99% *ee* from reaction of (*R*,*S*)-**4** with 25% ammonia in methanol.

### 2.7. Synthesis of (*R*,*S*,*S*,*S*)-**6**

Reaction of amino alcohol (*R*,*S*)-**5** and halohydrin (*S*,*R*)-**2** in methanol with potassium carbonate resulted in the formation of (−)-nebivolol ((*R*,*S*,*S*,*S*)-**6**) in 81% yield, with enantiomeric excess (*ee*) exceeding 99% and a global yield of 6% (Scheme 8). The reaction was conducted in methanol under reflux for 24 h, following a procedure adapted from Zhang et al. [21].




**Scheme 8.** Coupling of (*R*,*S*)-**5** and halohydrin (*S*,*R*)-**2** in methanol with potassium carbonate gave (−)-nebivolol ((*R*,*S*,*S*,*S*)-**6**) with 97% purity (HPLC and NMR), 81% reaction yield and >99% *ee*. The global yield of (*R*,*S*,*S*,*S*)-**6** was 6%.

HPLC analysis of (−)-nebivolol ((*R*,*S*,*S*,*S*)-**6**) was performed on a Chiralpak AD-H column with *n*-hexane and 2-propanol (85:15, *v*/*v*) as the mobile phase and a flow rate of 1 mL/min.; the retention time was $t_R$((*R*,*S*,*S*,*S*)-**6**) = 6.94 min. (Figure 10).

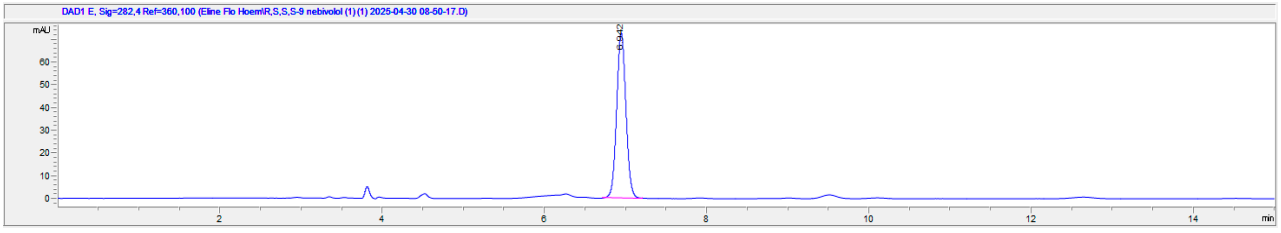



**Figure 10.** HPLC chromatogram of (−)-nebivolol ((*R*,*S*,*S*,*S*)-**6**) obtained using a Chiralpak AD-H column with *n*-hexane and 2-propanol (85:15, *v*/*v*) as the mobile phase, a flow rate of 1 mL/min., and a retention time of $t_R$((*R*,*S*,*S*,*S*)-**6**) = 6.94 min.

(*R*,*S*,*S*,*S*)-**6** was also analyzed on an achiral ZRBAX XDB-C18 column with water and acetonitrile (60:40, *v*/*v*) as the mobile phase and a flow rate of 1 mL/min. The retention time was $t_R$((*R*,*S*,*S*,*S*)-**6**) = 6.28 min. (Figure 11).

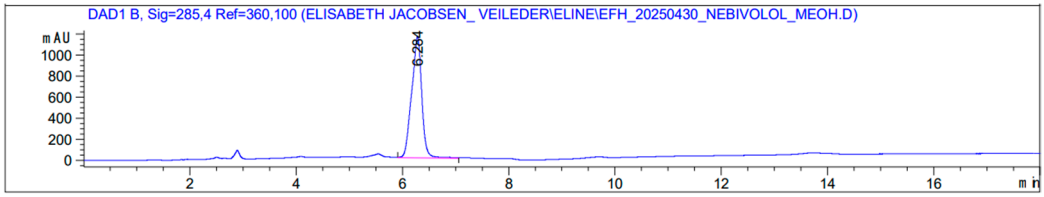



**Figure 11.** HPLC chromatogram of (−)-nebivolol ((*R*,*S*,*S*,*S*)-**6**) obtained on an achiral ZRBAX XDB-C18 column with water and acetonitrile (60:40, *v*/*v*) as the mobile phase, a flow rate of 1 mL/min., and a retention time of $t_R$((*R*,*S*,*S*,*S*)-**6**) = 6.28 min.

### *2.8. Specific Rotation Value of (−)-Nebivolol Hydrochloride ((R,S,S,S)-**6HCl**)*

(*R*,*S*,*S*,*S*)-**6** was transformed to its hydrochloride salt, (*R*,*S*,*S*,*S*)-**6HCl**, by dissolving it in methanol with subsequent addition of hydrochloric acid in dioxane. The observed specific rotation value of (*R*,*S*,*S*,*S*)-**6HCl** was $[\alpha]_D^{20} = -20°$ ($c = 1$, tetrahydrofuran:water, 4:1, *v*/*v*), in accordance with Tuchalski et al. (2006) [8] who reported a specific rotation value of their (*R*,*S*,*S*,*S*)-nebivolol hydrochloride of $[\alpha]_D^{29} = -20.5°$ ($c = 1$, tetrahydrofuran:water, 4:1, *v*/*v*). Identification of (*R*,*S*,*S*,*S*)-**6** and its hydrochloride salt was carried out by $^1$H, $^{13}$C, HSQC and HMBC NMR spectrometry using DMSO-$d_6$.

### *2.9. Specific Rotation Value of Halohydrins (R,R)-**2** and (S,R)-**2***

All four isomers of halohydrin (**2**) were isolated and characterized by $^1$H, $^{13}$C, HSQC, HMBC, NOESY and ROESY NMR spectrometry using DMSO-$d_6$. With the aim of reattaining halohydrins (*R*,*S*)-**2** and (*S*,*S*)-**2** after determination of kinetic resolution with CALB, esters (*R*,*S*)-**3** and (*S*,*S*)-**3** were hydrolyzed in separate pots in a phosphate buffer catalyzed by CALB. The reactions were performed at 30 °C and 200 rpm for 3 days. (*R*,*S*)-**2** was isolated in 33% yield and >99% *ee*, and (*S*,*S*)-**2** was isolated in 31% yield and >99% enantiomeric excess (*ee*). The reason for the low yields remains unknown, but since (*R*,*S*)-**2** and (*S*,*S*)-**2** are used in the synthesis of enantiopure (+)-nebivolol ((*S*,*S*,*S*,*R*)-nebivolol) and we are now focused on (−)-nebivolol synthesis, we have not focused on improving this synthesis step. A green aspect of this protocol is that hydrolysis of the esters **3** opens the possibility of synthesizing (+)-nebivolol in the same reaction as for (−)-nebivolol, (theoretically) resulting in no waste of the starting material, chloroketone **1.** Our observed specific rotation value of (*S*,*R*)-**2** is $[\alpha]_D^{20} = -80°$ ($c = 1$, MeOH), and for (*R*,*R*)-**2,** it is $[\alpha]_D^{20} = +86°$ ($c = 1$, MeOH). The same enantiomer as our (*R*,*R*)-**2** (NEB(*R*,*R*)) has been reported to have a specific rotation value of $[\alpha]_D^{20} = -89°$ ($c = 1$, $CHCl_3$) for 99% *ee* [20]. The opposite sign may be due to the use of different solvents. Xue et al. have not reported any specific rotation value for the nebivolol enantiomers.

## 3. Materials and Methods

### *3.1. Chemicals and Enzymes*

Chemicals and solvents used in this project were purchased from Sigma Aldrich (Oslo, Norway) and VWR (Oslo, Norway), while starting material **1** was sourced from TCI Co., Ltd. (Tokyo, Japan). Distilled water was prepared using a Barnstead Smart2Pure Pro Water Purification System (Thermo Scientific, Langenselbold, Germany). Molecular sieves (4Å) were dried at 1000 °C for 24 h and stored in a desiccator. Dry solvents were obtained from an MBraun MB-SPS-800 solvent purifier (Munich, Germany). All solvents used for HPLC analyses were of HPLC grade. *Candida antarctica* lipase B (CALB) expressed in *Pichia*

*pastoris* and immobilized on a hydrophobic polymer resin (>10,000 PLU, lot No.: 20170315) was kindly gifted by Syncozymes (Shanghai, China). *Candida antarctica* lipase B (CALB) immobilized on Immobead 150, recombinant from Aspergillus oryzae (≥1800 U/g, lot results: 4327 U/g, lot No.: unknown) from Sigma Aldrich, (Oslo, Norway). Enzymatic reactions were carried out in a New Brunswick G24 Environmental Incubator Shaker manufactured by Brunswick Co., Inc. (Edison, NJ, USA) at 37 °C and 200 rpm.

### *3.2. Optical Rotation Measurements and Absolute Configurations*

Optical rotation measurements were performed on an Anton Paar MCP 5100 polarimeter with a 10 mm long cell with 0.2 mL volume from Dipl. Ing. Houm AS (Oslo, Norway). Analyses were carried out at 20 °C at a wavelength of 589 nm (sodium D-line doublet). Absolute configuration of all isomers is based on our previously published work of CALB-catalyzed transesterifications of similar secondary alcohols, as presented here [11–18], and comparison of known specific rotation of the final enantiomer, i.e., (*S*,*R*,*R*,*R*)-**6HCL**, with the results reported by Tuchalski et al. (2006) [8].

### *3.3. Spectroscopic Analyses*

#### 3.3.1. NMR Spectroscopy

NMR analyses were recorded on a 600 MHz ($^1$H) Bruker Avance III UltraShield equipped with a CryoProbe 5 mm TCI probe head with *Z*-axis gradients from Bruker BioSpin GmbH (Karlsruhe, Germany) operating at 600 MHz for $^1$H, COSY, HSQC, and HMBC and 151 MHz for $^{13}$C NMR. The spectroscopic data were processed with Mestrelab Research software MestReNova 14.2.1. Chemical shifts (δ) are reported in parts per million (ppm) relative to TMS (or $CDCl_3$/DMSO-$d_6$ shifts), and coupling constants are reported in Hertz (Hz). Either $CDCl_3$ or DMSO-$d_6$ solvents were employed for sample preparation.

#### 3.3.2. Mass Spectrometry (MS)

Accurate mass determination in positive and/or negative mode was performed on a "Synapt G2-S" Q-TOF instrument from Waters$^{TM}$. It provides greater than 40,000 FWHM resolution and below 1 ppm mass accuracy for complex mixture analysis, structural characterization, and molecular imaging. A concentration of 200 ng/mL of leucine enkephalin was used as a Lockmass at a flow rate of 10 microliters per minute to allow for correction of exact mass measurements. Samples were ionized using an ASAP probe (APCI) or ESI probe. No chromatographic separation was used prior to the mass analysis. Exact mass calculation and spectra processing were done using Waters TM Software Masslynx V4.1 SCN871.

#### 3.3.3. Infrared Spectroscopy (IR)

Infrared spectroscopy (IR) spectra were recorded by an Alpha II FT-IR instrument from Bruker (Karlsruhe, Germany), and spectroscopic data were analyzed using OPUS 7.5 software. The spectral range was 400–4000 $cm^{-1}$ (Mid-IR), with a standard spectral resolution better than 2 $cm^{-1}$ (optional <0.75 $cm^{-1}$). Wavenumber accuracy was <0.05 $cm^{-1}$ at 2000 $cm^{-1}$, and wavenumber precision was <0.0005 $cm^{-1}$ at 2000 $cm^{-1}$ (10 repeated measurements). The signal-to-noise ratio (SNR) was typically >55,000:1 (1 min measurement, 4 $cm^{-1}$ resolution), and photometric accuracy was better than 0.1% T. The interferometer was a RockSolid™ instrument permanently aligned with a cube-corner mechanism, and the detector was a high-resolution DTGS.

### *3.4. Chromatographic Analyses*

#### 3.4.1. Thin-Layer Chromatography (TLC)

Thin-Layer Chromatography (TLC) analyses were performed on precoated TLC silica gel 60 F254 plates (0.2 mm) from Merck (Darmstadt, Germany). Visualization was done under ultraviolet light (254 nm) or with the use of phosphomolybdic acid.

#### 3.4.2. Column Chromatography

Column chromatography was performed on silica gel from Merck or VWR with a pore size of 60 Å (mesh particle size: 230–240; particle size: 40–63 μm).

#### 3.4.3. Chiral HPLC

Chiral HPLC analyses were conducted using a 1200 Quarternary LC system from Agilent Technologies (Santa Clara, CA, USA) equipped with an autosampler and a diode array detector (DAD) with a wavelength range of 190–950 nm and a 1024-element photodiode array to capture full spectra and multiple wavelengths simultaneously. Instrument control and data processing were managed by ChemStation software. C.01.07 The analyses maintained a flow rate of 1 mL/min with an injection volume of 1.5 μL. Detection was primarily performed at 220 and 254 nm; however, for (*R*,*S*,*S*,*S*)-**6**, detection was carried out at 282 nm.

Separation of all the isomers was performed on Daicel Chiralpak AD-H column (Lot No. ADH0CE-XI203, Part No. 19325) on silica gel (250 mm × 4.6 mm ID, 5 μm particle size) (Daicel, Chiral Technologies Europe, Gonthier d'Andernach, Illkirch, France), with *n*-hexane: *i*-PrOH as eluent (85:15, *v*/*v*) and a flow rate of 1 mL/min.: $t_R$((*S*,*R*)-**2**) = 6.31 min., $t_R$((*R*,*S*)-**2**) = 6.85 min., $t_R$((*S*,*S*)-**2**) = 7.96 min., $t_R$((*R*,*R*)-**2**) = 8.45 min., $t_R$((*R*,*S*)-**3**) = 4.14 min., $t_R$((*S*,*S*)-**3**) = 4.36 min., $t_R$((*S*,*R*)-**4**) = 4.58 min., $t_R$((*R*,*S*)-**4**) = 4.74 min., $t_R$((*S*,*S*)-**4**) = 4.99 min., $t_R$((*R*,*R*)-**4**) = 5.44 min., $t_R$((*R*,*S*)-**4**) = 6.25 min., and $t_R$((*R*,*S*,*S*,*S*)-**6**) = 6.94 min. Separation of **2** was also performed with: *n*-hexane: *i*-PrOH (90:10, *v*/*v*) as the eluent and a flow rate of 1 mL/min. $t_R$((*S*,*R*)-**2**) = 8.43 min., $t_R$((*R*,*S*)-**2**) = 9.21 min., $t_R$((*S*,*S*)-**2**) = 10.96 min., and $t_R$((*R*,*R*)-**2**) = 11.65 min. (Figure 2).

#### 3.4.4. Reverse-Phase HPLC

Reverse-phase HPLC analysis was performed on an Infinity 1290 Binary LC system from Agilent Technologies (Santa Barbara, CA, USA) equipped with an autosampler and a diode array detector (DAD) with a wavelength range of 190 to 640 nm, a data rate of up to 160 Hz, and very low noise levels. It utilizes Agilent Max-Light cartridge cell technology for enhanced sensitivity and baseline stability. Instrument control and data processing were managed by ChemStation software C.01.07. Analysis was conducted on a ZRBAX XDB-C18 column (150 mm Lx 4.6 mm ID; 5 μm), S.N. USKH112678, AGILENT. The injection volume was 5 μL. Detection was performed at 220, 254, and 285 nm.

Separation of (*R*,*S*,*S*,*S*)-**6** with $H_2O$:MeCN (60:40 *v*/*v*) as the eluent and a flow rate of 1 mL/min. gave $t_R$((*R*,*S*,*S*,*S*)-**6**) = 6.28 min.

### *3.5. Synthesis Protocols*

#### 3.5.1. Synthesis of 2-Chloro-1-(6-fluorochroman-2-yl)ethanone (**1**) from 6-Fluorochromane-2-carboxylic Acid

The synthesis of **1** was performed according to our previously reported procedure [10].

Yields of pure enantiomers are calculated based on the 50% maximum yield of each enantiomer, i.e., 70% yield is 70% of the 50% maximum yield.

3.5.2. Synthesis of 2-Chloro-1-(6-fluorochroman-2-yl)ethanol (**2**)

A mixture of $NaBH_4$ (0.09 g, 2.38 mmol) in EtOH (97%, 8 mL) was added slowly to a solution of **1** (0.36 g, 1.57 mmol) in EtOH (97%, 8 mL) at 0 °C. The mixture was stirred for 2.5 h. Full conversion was confirmed by TLC (*n*-hexane:EtOAc, 5:1); $R_f$ (**2**) = 0.23. The solution was poured into ice $H_2O$ (15 mL) before HCl in *i*-PrOH (1.25 M, 2.6 mL) was added to the mixture and stirred for 10 min. The aqueous phase was then extracted with $CH_2Cl_2$ (3 × 15 mL), and the combined organic layers were dried over anhydrous $MgSO_4$ and filtered. The solvent was removed under reduced pressure and further dried on a vacuum pump to yield the desired product, **2**, as a pale-yellow oil in 92% yield (0.33 g, 1.43 mmol) and 98% purity (HPLC). $^1$H NMR (600 MHz, DMSO-$d_6$) δ: 6.95–6.85 (m, 2H), 6.78–6.73 (m, 1H), 5.60 (d, *J* = 4.6 Hz, 0.5H), 5.47 (d, *J* = 5.3 Hz, 0.5H), 4.05 (ddd, *J* = 11.1, 3.2, 2.1 Hz, 0.5H), 3.91 (ddd, *J* = 9.6, 6.8, 2.4 Hz, 0.5H), 3.86–3.70 (m, 2H), 3.64 (td, *J* = 9.9, 6.1 Hz, 0.5H), 3.60–3.57 (m, 0.5H), 2.86–2.70 (m, 2H), 2.14 (m, 0.5H), 1.92 (m, 0.5H), 1.79 (m, 0.5H), 1.69 (m, 0.5H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ: 156.73, 150.70, 123.80, 117.41, 115.37, 113.75, 75.78, 71.40, 47.48, 23.59, 22.44. MS (TOF MS ASAP$^+$): *m*/*z* = 230.0509 $[M+H]^+$, (calc. mass $[M+H]^+$ = 230.0510, $C_{11}H_{12}O_2FCl$).

3.5.3. Synthesis of 6-Fluoro-2-(oxiran-2-yl)chroman (**4**)

$K_2CO_3$ (0.41 g, 3.00 mmol) was added to a stirred solution of racemic (**2**) (0.23 g, 1.00 mmol) in MeCN (10 mL). The solution was stirred at 50 °C for 17 h. Full conversion was confirmed by TLC (*n*-hexane:EtOAc, 5:1); $R_f$ ((*S*,*R*)-**4**/(*R*,*S*)-**4**) = 0.38, $R_f$ ((*R*,*R*)-**4**/(*S*,*S*)-**4**) = 0.50. $H_2O$ (10 mL) was added before the aqueous layer was extracted with $CH_2Cl_2$ (3 × 10 mL). The combined organic phases were washed with sat. NaCl (10 mL), dried over anhydrous $MgSO_4$, and filtered. The solvent was removed under reduced pressure and further dried on a vacuum pump to yield the desired product, **4**, as a yellow oil in 89% yield (0.17 g, 0.88 mmol) and >99% purity. $^1$H NMR (600 MHz, DMSO-$d_6$) δ: 6.96–6.86 (m, 2H), 6.78 (ddd, *J* = 8.9, 7.0, 4.9 Hz, 1H), 3.95 (ddd, *J* = 10.0, 4.8, 2.5 Hz, 0.5H), 3.75 (ddd, *J* = 10.4, 6.2, 2.4 Hz, 0.5H), 3.19–3.15 (m, 1H), 2.84–2.79 (m, 2H), 2.78–2.75 (m, 1H), 2.73 (ddd, *J* = 15.9, 5.1, 2.6 Hz, 1H), 2.04–1.97 (m, 1H), 1.83–1.67 (m, 1H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ: 155.20, 150.11, 123.63, 117.33, 115.42, 113.87, 76.58, 74.99, 53.12, 52.37, 44.54, 43.04, 23.42, 22.90. MS (TOF MS ASAP$^+$): *m*/*z* = 194.0741 $[M+H]^+$, (calc. mass $[M+H]^+$ = 194.0743, $C_{11}H_{11}O_2F$).

3.5.4. Synthesis of (*R*,*S*)-**4**

The same procedure was applied to (*R*,*R*)-**2** and (*S*,*R*)-**2** (0.19 g, 0.81 mmol, ratio 6.2:1), affording (*R*,*S*)-**4** and (*S*,*S*)-**4** (0.14 g, 0.72 mmol). (*R*,*S*)-**4** was separated from (*S*,*S*)-**4** by gradient column chromatography (*n*-hexane:EtOAc 100:0-40:60) to yield the desired product, (*R*,*S*)-**4**, as a pale-yellow oil in 69% yield (0.11 g, 0.57 mmol), >99% purity and >99% *ee*. $^1$H NMR (600 MHz, DMSO-$d_6$) δ: 6.95–6.87 (m, 2H), 6.79 (dd, *J* = 8.9, 4.9 Hz, 1H), 3.75 (ddd, *J* = 10.4, 6.2, 2.4 Hz, 1H), 3.17 (ddd, *J* = 6.2, 4.2, 2.7 Hz, 1H), 2.85–2.78 (m, 2H), 2.78–2.75 (m, 1H), 2.75–2.70 (m, 1H), 2.04–1.97 (m, 1H), 1.83–1.74 (m, 1H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ: 155.17, 150.12, 123.42, 117.33, 115.37, 113.69, 76.58, 53.12, 43.05, 23.67, 23.32.

3.5.5. Ring Opening of 6-Fluoro-2-(oxiran-2-yl)chroman (**4**)

Method 1: Ring Opening of Epoxides **4** by LiCl and AcOH

A stirred solution of (*S*,*R*)-**4**, (*R*,*S*)-**4** (0.65 g, 3.35 mmol), and LiCl (0.57 g, 13.40 mmol) in MeCN (20 mL) was treated with glacial AcOH (959 µL, 16.75 mmol). The solution was stirred at rt for 3 days. Full conversion was confirmed by TLC (*n*-hexane:EtOAc, 5:1) and HPLC. The reaction was quenched with sat. $NaHCO_3$ solution (5 mL) until a neutral pH

was achieved. $H_2O$ (10 mL) was added, and the aqueous mixture was extracted with $CH_2Cl_2$ (3 × 20 mL). The combined organic phase was washed with sat. NaCl (40 mL), dried over anhydrous $MgSO_4$, and filtered. The solvent was removed under reduced pressure and further dried on a vacuum pump to yield the desired products, (*S*,*S*)-**2** and (*R*,*R*)-**2**, as a pale-yellow oil in 93% yield (0.71 g, 3.09 mmol) and >99% purity. The same procedure was applied to (*S*,*S*)-**4** and (*R*,*R*)-**4** (0.49 g, 2.54 mmol), affording (*S*,*R*)-**2** and (*R*,*S*)-**2** (0.56 g, 2.43 mmol) as a yellow oil in 95% yield and >99% purity.

Method 2: Ring Opening of Epoxide **4** by HCl

HCl (37%, 0.5 mL) was added to a flask with a solution of racemic (**4**) (0.038 g, 0.20 mmol) and $CH_2Cl_2$ (1 mL). The reaction mixture was stirred at 0 °C. TLC (*n*-hexane:EtOAc, 5:1) showed full conversion of the substrate after 2–3 h. The organic phase was concentrated under reduced pressure and purified by gradient column chromatography (*n*-hexane:EtOAc, 100:0-40:60). This yielded the desired product, **2**, as a pale-yellow oil in 74% yield (0.031 g, 0.13 mmol) and >99% purity.

#### 3.5.6. Kinetic Resolution of 2-Chloro-1-(6-fluorochroman-2-yl)ethanol (**2**) with CALB

Activated molecular sieves (4 Å) and CALB (4.93 g, Sigma Aldrich) were added to a flask, which was purged with $N_2$ gas. (*R*,*R*)-**2**, (*S*,*S*)-**2** (0.71 g, 3.08 mmol), and vinyl butanoate (1.56 mL, 12.44 mmol) were then added, followed by anhydrous MeCN (40 mL). The reaction mixture was incubated at 37 °C and 200 rpm for 26 h before the enzyme and molecular sieves were filtered off, and the solvent was removed under reduced pressure. This yielded a crude mixture as a pale-brown oil. Halohydrin (*R*,*R*)-**2** and the produced ester, (*S*,*S*)-**3**, were separated by gravity column chromatography (*n*-hexane:EtOAc, 100:0-40:60); $R_f$ ((*R*,*R*)-**2**) = 0.23, $R_f$ ((*S*,*S*)-**3**) = 0.60. This yielded (*R*,*R*)-**2** as a pale-yellow oil in 71% yield (0.19 g, 0.82 mmol), >99% purity and 99% *ee*. (*S*,*S*)-**3** was obtained as a pale-yellow oil in 20% yield (0.05 g, 0.17 mmol), >99% purity, and 99% *ee*. The same procedure was applied to (*S*,*R*)-**2** and (*R*,*S*)-**2** (0.27 g, 1.17 mmol, ratio 9.6:1), affording (*S*,*R*)-**2** as a pale-yellow oil in 77% yield (0.15 g, 0.66 mmol mmol), >99% purity and 99% *ee*. (*R*,*S*)-**3** was obtained as a pale-yellow oil in 8% yield (0.016 g, 0.05 mmol), >99% purity and >99% *ee*.

#### 3.5.7. Characterization of (*S*,*R*)-**2**

$^1$H NMR (600 MHz, DMSO-$d_6$) δ: 6.95–6.85 (m, 2H), 6.76 (dd, *J* = 8.9, 4.9 Hz, 1H), 5.60 (d, *J* = 5.5 Hz, 1H), 3.91 (ddd, *J* = 9.6, 6.9, 2.4 Hz, 1H), 3.86–3.82 (m, 1H), 3.77–3.69 (m, 2H), 2.82–2.70 (m, 2H), 2.14 (dddd, *J* = 13.5, 6.0, 3.9, 2.4 Hz, 1H), 1.69 (dtd, *J* = 13.6, 10.2, 6.1 Hz, 1H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ: 155.95, 150.20, 123.75, 117.41, 115.37, 113.75, 75.78, 71.39, 47.48, 23.59, 22.44. $[\alpha]_D^{20} = -80°$ (*c* = 1, MeOH).

#### 3.5.8. Characterization of (*R*,*R*)-**2**

$^1$H NMR (600 MHz, DMSO-$d_6$) δ: 6.95–6.85 (m, 2H), 6.75 (dd, *J* = 8.9, 4.9 Hz, 1H), 5.47 (d, *J* = 6.3 Hz, 1H), 4.12–4.02 (m, 1H), 3.83–3.75 (m, 2H), 3.69–3.61 (m, 1H), 2.82 (ddd, *J* = 17.7, 12.4, 6.1 Hz, 1H), 2.74 (ddd, *J* = 16.7, 5.6, 2.4 Hz, 1H), 1.92 (ddt, *J* = 13.6, 6.1, 2.3 Hz, 1H), 1.79 (ddd, *J* = 24.6, 12.2, 5.5 Hz, 1H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ: 156.65, 155.09, 150.70, 123.71, 117.42, 115.29, 113.66, 76.00, 72.05, 45.94, 24.33, 22.43. $[\alpha]_D^{20} = 86°$ (*c* = 1, MeOH).

#### 3.5.9. Characterization of (*R*,*S*)-**3**

$^1$H NMR (600 MHz, DMSO-$d_6$) δ: 6.96–6.88 (m, 2H), 6.78 (dd, *J* = 8.9, 4.9 Hz, 1H), 5.20–5.17 (m, 1H), 4.20 (ddd, *J* = 10.2, 6.2, 2.4 Hz, 1H), 4.00 (dd, *J* = 12.0, 3.2 Hz, 1H), 3.90 (dd, *J* = 12.1, 7.1 Hz, 1H), 2.81 (ddd, *J* = 17.1, 10.9, 5.8 Hz, 1H), 2.77–2.69 (m, 1H), 2.36 (t, *J* = 7.2 Hz, 2H), 2.04 (ddt, *J* = 13.6, 6.0, 3.0 Hz, 1H), 1.68 (dtd, *J* = 13.6, 11.1, 5.6 Hz, 1H), 1.58 (h, *J* = 7.3 Hz, 2H), 0.89 (t, *J* = 7.4 Hz, 3H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ: 172.07,

156.93, 149.80, 123.60, 117.51, 115.39, 113.90, 73.98, 72.70, 43.48, 35.31, 23.47, 22.19, 17.99, 13.32. MS (TOF MS ASAP$^+$): $m/z$ = 301.1009 [M+H]$^+$, (calc. mass [M+H]$^+$ = 301.1007, $C_{15}H_{19}NO_3FCl$).

#### 3.5.10. Characterization of (*S*,*S*)-**3**

$^1$H NMR (600 MHz, DMSO-d$_6$) δ: 6.95–6.88 (m, 2H), 6.75 (dd, *J* = 8.9, 4.9 Hz, 1H), 5.23 (dt, *J* = 8.0, 4.1 Hz, 1H), 4.25 (ddd, *J* = 10.7, 4.0, 2.2 Hz, 1H), 3.99 (dd, *J* = 11.7, 4.2 Hz, 1H), 3.89 (dd, *J* = 11.7, 7.9 Hz, 1H), 2.83 (ddd, *J* = 17.4, 11.7, 6.1 Hz, 1H), 2.73 (ddd, *J* = 16.9, 5.6, 2.8 Hz, 1H), 2.36 (td, *J* = 7.2, 1.8 Hz, 2H), 1.93 (ddt, *J* = 13.5, 5.7, 2.5 Hz, 1H), 1.66 (dtd, *J* = 13.5, 11.7, 5.5 Hz, 1H), 1.58 (h, *J* = 7.3 Hz, 2H), 0.91 (t, *J* = 7.4 Hz, 3H). $^{13}$C NMR (151 MHz, DMSO-d$_6$) δ: 172.29, 156.86, 150.06, 123.46, 117.50, 115.36, 113.70, 74.30, 73.06, 43.20, 35.26, 23.82, 22.55, 18.04, 13.36.

#### 3.5.11. Hydrolysis of 2-Chloro-1-(6-fluorochroman-2-yl)ethyl Butanoate (**3**) Using CALB

(*S*,*S*)-**3**, (*R*,*S*)-**3** (0.030 g, 0.10 mmol), and CALB (0.10 g, Syncocymes Co. LTD, Shanghai, China) were added to a phosphate-buffered solution (0.1 M, pH 7.0, 7 mL). The mixture was incubated at 30 °C and 200 rpm for 3 days before the mixture was filtered and the filtrate was extracted with EtOAc (3 × 10 mL). The organic phases were dried over anhydrous $MgSO_4$ and filtered, and the solvent was removed under reduced pressure to yield (*S*,*S*)-**2** and (*R*,*S*)-**2** in 40% yield (9.07 mg, 0.04 mmol) and 97% purity as a pale-yellow oil.

#### 3.5.12. Hydrolysis of (*R*,*S*)-**3** to Give (*R*,*S*)-**2**

Following the procedure described above, (*R*,*S*)-**3** (0.027 g, 0.09 mmol, >99% *ee*) was converted to (*R*,*S*)-**2** as a pale-yellow oil in 33% yield (6.8 mg, 0.03 mmol), >99% purity and >99% *ee*. $^1$H NMR (600 MHz, DMSO-d$_6$) δ: 6.93–6.86 (m, 2H), 6.75 (dd, *J* = 8.8, 4.9 Hz, 1H), 5.47 (d, *J* = 6.2 Hz, 1H), 4.07–4.02 (m, 1H), 3.83–3.75 (m, 2H), 3.68–3.61 (m, 1H), 2.82 (dddd, *J* = 16.9, 12.3, 6.1, 1.2 Hz, 1H), 2.74 (ddd, *J* = 16.7, 5.5, 2.4 Hz, 1H), 1.92 (ddt, *J* = 13.6, 6.0, 2.3 Hz, 1H), 1.79 (dddd, *J* = 13.5, 12.2, 11.0, 5.5 Hz, 1H). $^{13}$C NMR (151 MHz, DMSO-d$_6$) δ: 156.65, 150.71, 123.71, 117.43, 115.30, 113.67, 76.00, 72.05, 45.95, 24.34, 22.43.

#### 3.5.13. Hydrolysis of (*S*,*S*)-**3** to Give (*S*,*S*)-**2**

Following the procedure described above, (*S*,*S*)-**3** (0.021 g, 0.07 mmol, >99% *ee*) was converted to (*S*,*S*)-**2** as a pale-yellow oil in 31% yield (5.0 mg, 0.02 mmol), 93% purity and >99% *ee*. $^1$H NMR (600 MHz, DMSO-d$_6$) δ: 6.94–6.86 (m, 2H), 6.76 (dd, *J* = 8.9, 4.9 Hz, 1H), 5.60 (d, *J* = 5.4 Hz, 1H), 3.91 (ddd, *J* = 9.6, 6.9, 2.4 Hz, 1H), 3.86–3.82 (m, 1H), 3.75–3.70 (m, 2H), 2.79–2.73 (m, 2H), 2.17–2.11 (m, 1H), 1.69 (dtd, *J* = 13.6, 10.2, 6.1 Hz, 1H). $^{13}$C NMR (151 MHz, DMSO-d$_6$) δ: 156.73, 150.20, 123.80, 117.41, 115.38, 113.75, 75.78, 71.39, 47.48, 23.59, 22.44.

#### 3.5.14. Synthesis of (*S*)-2-Amino-1-((*R*)-6-fluorochroman-2-yl)ethanol ((*R*,*S*)-**5**)

A solution of aqueous ammonia (25%, 9 mL, 0.125 mmol) and MeOH (10 mL) was cooled to 10–15 °C before a solution of (*R*,*S*)-**4** (0.080 g, 0.41 mmol) in MeOH (10 mL) was added over 2 h. The reaction mixture was stirred at 10–15 °C for 4 h, then gradually warmed to 25–35 °C and stirred for 3 h. To drive the reaction to completion, aqueous ammonia (25%, 2 mL, 28 mmol) was added, and the reaction was stirred overnight at rt. A further addition of aqueous ammonia (25%, 1 mL, 14 mmol) was added, and the mixture was heated to 35 °C and stirred for 3 h. Full conversion was confirmed by TLC (*n*-hexane:EtOAc, 5:1). MeOH was removed under reduced pressure, and cold $H_2O$ (10 mL) was added. The aqueous layer was extracted with $CH_2Cl_2$ (3 × 20 mL), and the combined organic layers were washed with sat. NaCl solution (20 mL), dried over anhydrous $MgSO_4$, and filtered. The solvent was removed under reduced pressure and dried on a vacuum pump to yield

the desired product, (*R*,*S*)-**5**, as a white powder in 79% yield (0.069 g, 0.33 mmol), >99% purity and >99% *ee*. $^{1}$H NMR (600 MHz, DMSO-$d_6$) δ: 6.92–6.83 (m, 1H), 6.72 (dd, *J* = 8.9, 4.9 Hz, 1H), 4.80 (s, 1H), 3.94 (ddd, *J* = 10.9, 4.1, 2.1 Hz, 1H), 3.46 (dt, *J* = 8.2, 4.4 Hz, 1H), 2.83–2.75 (m, 1H), 2.75–2.68 (m, 2H), 2.65–2.59 (m, 1H), 1.93–1.85 (m, 1H), 1.77–1.67 (m, 1H), 1.49 (s, 2H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ: 156.51, 154.95, 151.02, 123.78, 117.35, 115.26, 113.41, 77.10, 73.67, 43.72, 24.50, 22.59. MS (TOF MS ASAP$^+$): *m*/*z* = 212.1089 [M+H]$^+$, (calc. mass [M+H]$^+$ = 212.1087, $C_{11}H_{15}NO_2F$).

3.5.15. Synthesis of (−)-Nebivolol ((*R*,*S*,*S*,*S*)-**6**)

(*R*,*S*)-**5** (0.055 g, 0.24 mmol) and $K_2CO_3$ (0.033 g, 0.24 mmol) were added to a stirred solution of (*S*,*R*)-**2** (0.051 g, 0.24 mmol) in MeOH (4.5 mL). The mixture was stirred under reflux (75 °C) for 24 h before the solvent was removed under reduced pressure. Cold $H_2O$ was added, the flask was rinsed with $CH_2Cl_2$ to transfer the organic material, and the aqueous layer was extracted with $CH_2Cl_2$ (3 × 10 mL). The combined organic phase was washed with cold $H_2O$ (2 × 10 mL) and sat. NaCl (10 mL), dried over anhydrous $MgSO_4$, and filtered. The solvent was removed under reduced pressure, and the residue was dried on a vacuum pump to yield the desired product, (*R*,*S*,*S*,*S*)-**6**, as a white powder in 81% yield (0.079 g, 0.19 mmol), 97% purity (HPLC) and >99% *ee*. $^{1}$H NMR (600 MHz, DMSO-$d_6$) δ: 6.92–6.81 (m, 4H), 6.74–6.65 (m, 2H), 5.10 (d, *J* = 5.6 Hz, 0.5H), 5.00 (d, *J* = 5.3 Hz, 0.5H), 4.84 (d, *J* = 3.7 Hz, 0.5H), 4.72 (d, *J* = 4.4 Hz, 0.5H), 3.96–3.84 (m, 2H), 3.76–3.68 (m, 1H), 3.68–3.60 (m, 1H), 3.28 (s, 1H), 2.83–2.56 (m, 8H), 2.12–1.95 (m, 1H), 1.95–1.83 (m, 1H), 1.78–1.71 (m, 1H), 1.71–1.64 (m, 1H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ: 155.01, 150.96, 123.78, 117.31, 115.18, 113.58, 77.42, 70.52, 69.23, 58.48, 51.37, 24.47, 23.83, 22.30, 21.94. MS (TOF MS ASAP$^+$): *m*/*z* = 406.1836 [M+H]$^+$, (calc. mass [M+H]$^+$ = 406.1830, $C_{22}H_{25}NO_4F_2$).

3.5.16. Synthesis of (−)-Nebivolol Hydrochloride ((*R*,*S*,*S*,*S*)-**6HCl**)

(*R*,*S*,*S*,*S*)-**6** (0.045 g, 0.11 mmol) was dissolved in MeOH (1 mL), and HCl in dioxane (4 M, 27.6 µL) was added. The solution was stirred at rt for 1 h. The pH was confirmed to be acidic. It was kept in an ice bath for 1 h, then in the fridge overnight, with no tendency to crystallize. Therefore, the solvent was removed under reduced pressure. The crude product was redissolved in EtOAc (1 mL), and cold $Et_2O$ (1 mL) was added dropwise while stirring with a glass rod in an ice bath. Crystallization occurred, and the crystals were collected by suction filtration, washed with cold $Et_2O$ (1–2 mL), and dried on a vacuum pump to yield the desired product, (*R*,*S*,*S*,*S*)-**6HCl**, as a white salt in 68% yield (0.033 g, 0.07 mmol) and >99% *ee*. $^{1}$H NMR (600 MHz, DMSO-$d_6$) δ: 8.63 (d, *J* = 35.9 Hz, 1H), 7.02–6.85 (m, 4H), 6.80–6.72 (m, 2H), 6.01 (d, *J* = 6.1 Hz, 0.5H), 5.95 (d, *J* = 5.7 Hz, 0.5H), 5.76 (d, *J* = 5.7 Hz, 0.5H), 5.67 (d, *J* = 5.8 Hz, 0.5H), 4.17–4.06 (m, 1H), 4.05–3.98 (m, 1H), 3.98–3.91 (m, 1H), 3.91–3.83 (m, 1H), 3.75–3.66 (m, 1H), 3.60–3.43 (m, 1H), 3.26–3.13 (m, 1H), 3.10–2.98 (m, 1H), 2.87–2.73 (m, 4H), 2.20–2.08 (m, 1H), 2.03–1.89 (m, 1H), 1.85–1.72 (m, 1H), 1.74–1.63 (m, 1H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ: 155.18, 150.48, 123.79, 117.42, 115.39, 113.75, 76.94, 67.40, 49.64, 24.14, 23.54, 22.38, 22.22. $[\alpha]_D^{20} = -20°$ (*c* = 1, THF:$H_2O$, 4:1).

## 4. Conclusions

We successfully synthesized (−)-nebivolol ((*R*,*S*,*S*,*S*)-nebivolol, (*R*,*S*,*S*,*S*)-**6**) in 97% purity and >99% *ee* by using a CALB-catalyzed enantioselective route that eliminated the need for preparative HPLC. This protocol is also a shorter and more efficient route to (−)-nebivolol than previously reported protocols. Halohydrins (**2**) were converted into epoxides (**4**), which were separated by column chromatography and subsequently ring-opened to regenerate the corresponding halohydrins (**2**) for CALB-catalyzed kinetic resolution. Enantiopure intermediates (*S*,*R*)-**2** and (*R*,*S*)-**4** were then coupled via amination

of (*R*,*S*)-**4** to amino alcohol (*R*,*S*)-**5**, affording (−)-nebivolol. Finally, (−)-nebivolol was converted to its hydrochloride salt, (*R*,*S*,*S*,*S*)-**6HCl**, in a global yield of 6% (81% step yield) exhibiting a specific rotation of $[\alpha]_D^{20} = -20^\circ$ ($c = 1$, tetrahydrofuran:water, 4:1, $v/v$), in agreement with the literature [8]. The absolute configuration was confirmed by CALB selectivity, chiral HPLC, and NMR analyses. Specific rotation values of the key precursors, (*S*,*R*)-**2** and (*R*,*R*)-**2**, were determined as $[\alpha]_D^{20} = -80^\circ$ ($c = 1$, MeOH) and $[\alpha]_D^{20} = +86^\circ$ ($c = 1$, MeOH), respectively. Hydrolysis of the enantiomerically pure butanoate esters (**3**) from the kinetic resolution of the four halohydrins (the two "wrong" building blocks for (−)-nebivolol) can be used for production of (+)-nebivolol, thereby utilizing 100% of the starting material, in accordance with the Green Chemistry Principles outlined by Paul Anastas and John Warner (1998) [22]. By using enzymes as catalysts, the reaction temperature can be kept under 40°, saving energy. This is favorable both for an industrial process and for the environment. The EU Green Deal aims to cut $CO_2$ emissions, in addition to focusing on more energy-efficient products, as our protocols for nebivolol enantiomers surely are. The global yield of (*R*,*S*,*S*,*S*)-**6HCl** will be improved.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/catal16030256/s1.

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