Tetrahedron: *Asymmetry* 19 (2008) 2439–2442

Contents lists available at ScienceDirect

Tetrahedron: *Asymmetry*

journal homepage: www.elsevier.com/locate/tetasy

Chemoenzymatic synthesis of the potential antihypertensive agent (2*R*,2′*S*)-β-hydroxyhomometoprolol

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ARTICLE INFO

Article history:
Received 25 June 2008
Accepted 5 November 2008
Available online 29 November 2008

ABSTRACT

The kinetic resolution of 1-chloro-3-[4-(2-methoxyethyl)phenoxy]-2-propanol *rac*-**4** with Novozym 435 and vinyl stearate, a key step in the gram-scale synthesis of (2*S*)-2-[[(2*R*)-2-hydroxy-3-[4-(2-methoxyethyl)phenoxy]propyl]amino]-1-butanol (*R*,*S*)-**1** a potent antihypertensive agent currently under investigation, is reported here. Our approach differs from the previously reported synthesis, which involves a tedious and poorly effective fractional crystallization of (*R*,*S*)-**1**. This novel approach incorporates an enzymatic resolution for the efficient preparation of the oxirane precursor (*R*)-**3**. The two main advantages arising from this strategy are the high enantioselectivity of the enzymatic process and the facilitated recovery of the hydrophobic stearate intermediate (*S*)-**5**.

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1. Introduction

In the search for new antihypertensive drugs with better therapeutic potential and fewer side effects, we recently reported the synthesis and pharmacological evaluation of four new analogues of Metoprolol with an additional stereogenic center (Fig. 1).[1]

Although these analogues did not cause β_1-adrenergic receptor inhibition, the diastereomer with (*R*)-configuration at the secondary alcohol center and an (*S*)-configuration at C-2 of the nitrogen chain (*R*,*S*)-**1** showed significant, but short-lasting hypotension as well as a modest and short-lasting bradychardia, suggesting antiarrhythmic activity. This compound, (2*S*)-2-[[(2*R*)-2-hydroxy-3-[4-(2-methoxyethyl)phenoxy]propyl]amino]-1-butanol (*R*,*S*)-**1,** was tentatively named (2*R*,2′*S*)-β-hydroxyhomometoprolol (Fig. 1).

The first step in the previously reported synthesis of the new compounds (*R*,*S*)-**1** and (*S*,*S*)-**1** involves the reaction between the

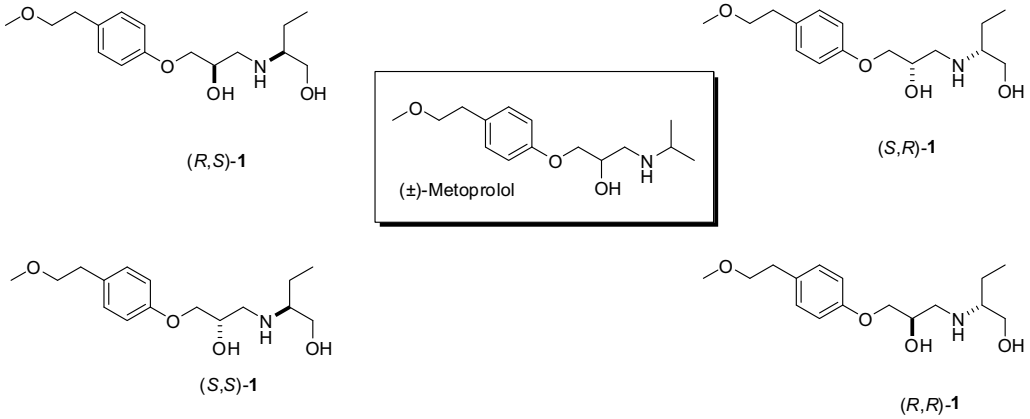

Figure 1. Structure of metoprolol and β-hydroxyhomometoprolol diastereomers.

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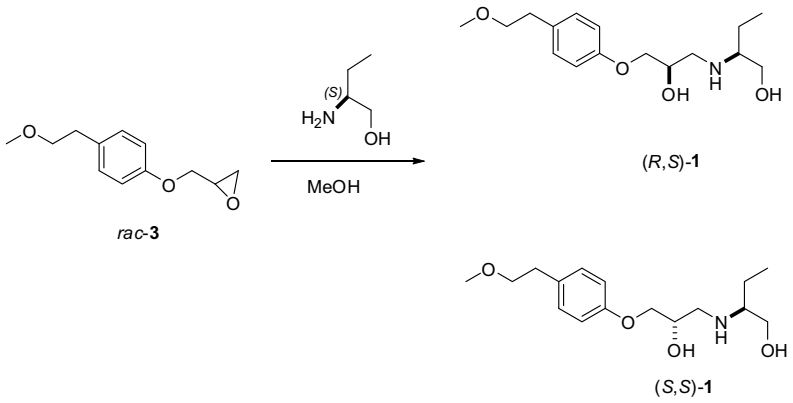
doi:10.1016/j.tetasy.2008.11.003



Scheme 1. Previously reported chemical synthesis of (*R*,*S*)-**1** and (*S*,*S*)-**1**.[1]

racemic epoxide (±)2-((4-(2-methoxyethyl)phenoxy)methyl)oxirane *rac*-**3** and (*S*)-2-amino-1-butanol (Scheme 1).

While the preparation of these compounds seems straightforward, it requires the separation of the diastereomeric products (*R*,*S*)-**1** and (*S*,*S*)-**1** via a tedious fractional crystallization. Furthermore, the desired diastereomer (*R*,*S*)-**1** remains in the mother liquors, and is isolated in reduced yields (21% yield from *rac*-**3**).

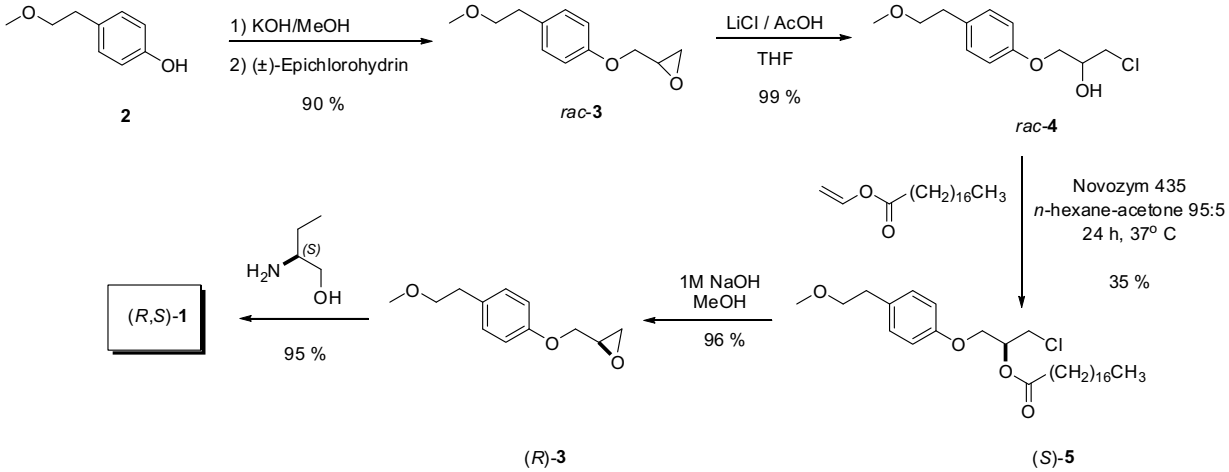
Over the last few years, enzymes in organic media have become a useful tool in organic synthesis due to their chemo-, regio-, and enantioselective properties. Due to their high stability and catalytic promiscuity, particular attention has been paid to lipases (triacylglycerol hydrolases, EC 3.1.1.3), since they have been shown to be suitable enzymes for the catalysis of a wide variety of chemical reactions.[2] Furthermore, because of the high enantioselectivity of the enzymatic process, they have been used extensively in the resolution of alcohols, esters, carboxylic acids, amino acids, amines, and amides.[3,4] One of the most commonly used lipases in organic syntheses is lipase B from *Candida antarctica* (CaLB), which has an operational limit of up to 80 °C in its immobilized form (Novozym® 435) and works efficiently in different organic solvents, as it is highly resistant to denaturalization.[5–7] According to Kazlauskas' rules,[8] the steric effect of the substrate is a decisive factor in the degree of enantioselectivity in acylation reactions of chiral secondary alcohols; under this consideration, CaLB should have a preference for the (*R*) enantiomer.[9]

In order to carry out detailed pharmacologic and pharmacokinetic studies of (*R*,*S*)-**1**, a more efficient gram-scale chemoenzymatic synthesis of this compound was developed. This new strategy is based on elimination of the previously reported fractional crystallization step required for the separation of diastereomers (*R*,*S*)-**1** and (*S*,*S*)-**1**. As an alternative, we introduced a chemoenzymatic step that would lead to the preparation of the oxirane enantiomer (*R*)-**3** in good enantiomeric excess. From this enriched precursor, the addition of (*S*)-2-amino-1-butanol directly provides diastereomer (*R*,*S*)-**1** (Scheme 2).

2. Results and discussion

In the first step, the synthesis of 2-[4-(2′-methoxyethyl)-phenoxymethyl]-oxirane *rac*-**3** was carried out by treatment of the potassium salt of 4-(2-methoxyethyl)phenol **2** with an excess of (±)-epichlorohydrin (90% yield). The racemic chlorohydrin *rac*-**4** was then obtained by the regioselective ring-opening of *rac*-**3** with lithium chloride and acetic acid, in a nearly quantitative yield (99%).[10]

In this step, we introduced the enzymatic resolution of *rac*-**4**, by taking advantage of the reported (*R*)-specificity of lipase Novozym® 435 during alcoholysis reactions.[9] An additional advantage was introduced using a hydrophobic acyl moiety that allows a facilitated separation of the hydrophobic enantiomer (*S*)-**5** from the non-reacted polar (*R*)-**4**. Accordingly, *rac*-**4** was reacted at 37 °C with Novozym® 435, and the vinyl esters of acetic, lauric, oleic, and stearic acids were used as acyl donors in 95:5 hexane–acetone as solvent. All reactions were stopped after 28 h, in a compromise between yield and ee, as lower conversions resulted in better ee but modest yields of (*S*)-**5**. After these reaction times, the desired (*S*)-chlorohydrin esters were recovered and separated



Scheme 2. Chemoenzymatic synthesis of (2*R*,2′*S*)-β-hydroxyhomometoprolol, (*R*,*S*)-**1**.

by column chromatography. It is important to note that in contrast to other acyl residues traditionally tested in this type of resolution,[11] the introduction of a stearyl residue was more advantageous. Actually, the hydrophobic stearate (*S*)-**5** was easily separated after column chromatography due to its high solubility in *n*-hexane–acetone (35.4% yield; theoretical maximum yield of 50%). Compound (*S*)-**5** was easily recovered as a white crystalline product, which was efficiently transesterified by treatment with 1 equiv of NaOH in MeOH to provide the desired (*R*)-**3** in 96% yield and 94.7% ee, along with the corresponding methyl stearate. Finally, the nucleophilic opening of this epoxide with (*S*)-2-amino-1-butanol led directly to the desired diastereomeric amino alcohol (*R*,*S*)-**1** in 95% yield.

3. Conclusion

The global process involving the chemoenzymatic synthesis of target compound (*R*,*S*)-**1** resulted in a 31% overall yield from the racemic oxirane precursor *rac*-**3**. For the most part, this strategy was successful because of the high enantioselectivity exhibited by Novozym® 435 lipase and the facile recovery of (*S*)-**5** which, after transesterification and reaction with (*S*)-2-aminobutanol, provided an easily recrystallized (*R*,*S*)-**1**, leaving only a small amount of unwanted diastereomer (*S*,*S*)-**1** in the mother liquors. This chemoenzymatic approach completely avoids the use of the inefficient fractional crystallization for (*R*,*S*)-**1** involved in the chemical syntheses previously reported.

4. Experimental

4.1. Materials and instruments

Lipase B from *Candida antarctica* was used in its immobilized form Novozym® 435. The reagents were purchased from Aldrich Chemical Company. Reactions were monitored by TLC using silica gel as the stationary phase and visualized using UV irradiation 254/366 nm and iodine vapors. In the chromatographic columns, silica gel was used as the stationary phase 70–230 mesh. The enzymatic resolution was conducted in a Shaker bath orbital incubator.

^{1}H and ^{13}C NRM were determined in a JEOL Eclipse instrument at 300 and 400 MHz, using tetramethylsilane as an internal standard and $CDCl_3$ as a solvent. The infrared spectra were obtained in a Bruker spectrometer model Tensor 27. Optical rotations were determined in a Perkin Elmer polarimeter model 341 using a 1 dm cell length. Measurements were done using the sodium D-line (589 nm) at a sample compartment temperature of 20 °C. The mass spectra were determined in a JEOL instrument model JMS-SX102A. Elemental analyses were obtained with a Exeter Analytical CE-440 apparatus.

4.2. Chiral HPLC analysis

Enantiomers of 2-{[4-(2-methoxyethyl)phenoxy] methyl}oxirane were quantified in a CHIRALCEL OB-H column (4.6 × 250 mm) (Chiral Technologies Inc., West Chester PA, USA), using a Waters 600E system controller and a Waters 996 photodiode array detector at 224 nm (Waters Copr. Milford, MA, SA). The effluent was *n*-hexane–ethanol 90:10 (v/v) at a flow rate of 1 ml min^{-1}. The retention time for the (*R*)-**3** isomer is 16.2 min and 19.5 min for the (*S*)-**3** enantiomer.

4.2.1. (±)-2-{[4-(2-Methoxyethyl)phenoxy]methyl}oxirane *rac*-3

In a 2 L three-necked flask, fitted with a mechanical stirrer, a condenser, and a thermometer, a solution of 100 g (657 mmol) of 4-(2-methoxyethyl)phenol **2** in 200 mL MeOH was placed. To this solution, 45 g (683 mmol) of 85% KOH was added, and the mixture was stirred for 1 h at 40–45 °C. Afterwards, 150 mL of MeOH was distilled off at atmospheric pressure, and 1 L epichlorohydrin was added to the semisolid residue. After 24 h of stirring at 40 °C and TLC monitoring (CH_2Cl_2–AcOEt 95:5), the mixture was washed with water (3 × 100 mL, pH 7.5), dried over anhydrous Na_2SO_4, and vacuum distilled in order to recover excess epichlorohydrin (190 mmHg/70 °C). Toluene (120 mL) was added to the residue, and the mixture was distilled again at 75 mmHg/40 °C until constant weight to obtain 147.11 g of a mixture containing *rac*-**3** (90%) and *rac*-**4** (10%).[1,12]

4.2.2. (±)-1-[4-(2-Methoxyethyl)phenoxy]-3-chloro-2-propanol *rac*-4

To a solution of *rac*-**3** (30 g (144.1 mmol)) in 250 mL THF, 19.78 mL (432.46 mmol, 3 equiv mol) acetic acid and 9.78 g (230.4 mmol, 1.6 equiv mol) LiCl were added. The reaction mixture was stirred at room temperature for 48 h. After TLC verification of the end of the reaction (CH_2Cl_2–AcOEt 95:5), the reaction mixture was concentrated under reduced pressure and partitioned in 300 mL water and 150 mL AcOEt. The organic layer was washed with saturated $NaHCO_3$ until free from acid, dried with anhydrous Na_2SO_4, and concentrated in a rotary evaporator to yield 34.88 g of pure product as a colorless oil in 99% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.82 (t, J = 6.9 Hz, 2H), 3.34 (s, 3H), 3.56 (t, J = 7.2 Hz, 2H), 3.66 (dd, J_1 = 11.4, J_2 = 6 Hz, 1H), 3.73 (dd, J_1 = 11.4, J_2 = 5.1 Hz, 1H), 3.99 (dd, J_1 = 6.3, J_2 = 1.2 Hz, 1H), 4.05 (dd, J_1 = 6, J_2 = 1.2 Hz, 1H), 4.17 (quintet, J = 10.5 Hz, 1H), 6.81 (d, J = 9 Hz, 2H), 7.11 (d, J = 9 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 35.1, 45.8, 58.5, 68.5, 69.7, 73.6, 114.4, 129.7, 131.7, 156.6. Lit.[13,14]

4.2.3. (2*S*)-1-Chloro-3-[4-(2-methoxyethyl)phenoxy]-2-propanol-2-stearate (*S*)-5

In a tightly closed vessel, 12 g (49.1 mmol) of *rac*-**4** was dissolved in a mixture of 360 mL hexane and 20 mL acetone. To this solution, 4.5 g of 4 Å molecular sieves and 8 g (25.7 mmol, 0.5 equiv mol) of vinyl stearate were added (a 60 μL aliquot was saved as a reference) together with 3.8 g of the biocatalyst Novozym 435. This mixture was incubated for 28 h a 37 °C with orbital stirring at 200 rpm. Afterwards, the mixture was filtered through Celite and concentrated under reduced pressure to yield 19.5 g of a crude product, which after separation in a chromatographic column in 370 g of silica gel (hex–AcOEt 95:5) rendered 8.85 g (35.4%) of (*S*)-**5** as white crystals with a mp 42–43 °C and $[\alpha]_D^{20} = +13.6$ (*c* 1, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.87 (t, J = 6.9 Hz, 3H), 1.25 (s, 14H), 1.63 (quintet, J = 7.2 Hz, 2H), 2.30–2.38 (m, 2H), 2.82 (t, J = 6.9 Hz, 2H), 3.34 (s, 3H), 3.56 (t, J = 7.2 Hz, 2H), 3.73–3.87 (m, 2H), 4.09–4.18 (m, 2H), 5.32 (quintet, J = 10.2 Hz, 1H), 6.82 (d, J = 9 Hz, 2H), 7.12 (d, J = 9 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.0, 22.6, 24.7, 24.8, 29.0, 29.2, 29.3, 29.4, 29.5, 29.6, 31.8, 31.9, 33.7, 34.2, 35.2, 42.4, 42.5, 58.6, 66.0, 70.8, 73.7, 114.5, 129.8, 131.8, 156.7, 173.0. IR υ_{max} (KBr) cm^{-1}: 2928, 2850, 1738, 1702, 1467, 1246, 1168, 944. EM (IE) m/z (%) M$^+$: 511 (2.5), 359 (100), 331 (3), 244 (4), 147 (7.5), 107 (7), 71 (7.5), 57 (12), 43 (11). Elemental Anal. Calcd for $C_{30}H_{51}ClO_4$ (511.18): C, 70.49; H, 10.06. Found: C, 70.50; H, 9.84.

4.2.4. (*R*)-2-{[4-(2-Methoxyethyl)phenoxy]methyl}oxirane (*R*)-3

16.2 mL of 1 M NaOH (1 equiv mol) was added to a solution containing 8.3 g (16.26 mmol) of (*S*)-**5** in 400 mL MeOH (ultrasound-assisted solution) and stirred for 24 h at room temperature, while monitoring the reaction progress by TLC (hex–AcOEt 85:15). After completion, the reaction medium was cooled to 0–5 °C for

1 h, and the solids were vacuum filtered and washed with 75 mL of cold MeOH. The filtrate was evaporated under reduced pressure, and the remaining oily liquid (5.5 g) was purified by column chromatography in silica gel (1. hex–AcOEt 95:5, 2. hex–AcOEt 85:15) to yield 3.23 g of (*R*)-**3** in 96% yield and 94.7% ee. $[\alpha]_D^{20} = -11.3$ (*c* 1, MeOH).

4.2.5. (2*R*,2′*S*)-β-Hydroxyhomometoprolol (*R*,*S*)-1

To a solution of (*R*)-**3** (2 g; 9.6 mmol) in 14 mL MeOH, 5.81 g (65.17 mmol, 6.7 M equiv) of (*S*)-2-amino-1-butanol was added. After 24 h of stirring and TLC monitoring (CH_2Cl_2–AcOEt 95:5), the reaction mixture was evaporated under reduced pressure and partitioned in 20 mL $CHCl_3$ and 20 mL of water. After drying the organic extract and evaporating the solvent, the remaining solid (3.1 g) was recrystallized from toluene to yield 2.66 g (95%) of (*R*,*S*)-**1**, mp 74–75 °C, $[\alpha]_D^{20} = +10.3$ (*c* 1, MeOH). Lit[1] mp 74–75 °C, $[\alpha]_D^{20} = +10.3$ (*c* 1, MeOH).

Acknowledgments

The authors acknowledge DGAPA-UNAM for Ignacio Regla's sabbatical fellowship at IBT-UNAM. Authors are also grateful to María de los Angeles Peña, Elizabeth Huerta, Erendira García Rios, Javier Pérez-Flores and Rocío Patiño-Maya from Instituto de Química, UNAM for spectroscopic analysis and to Novozymes-México for the generous gift of CaLB.

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