Pergamon

Tetrahedron: Asymmetry, Vol. 7, No. 7, pp. 2017-2022, 1996

0957-4166/96 $15.00 + 0.00

PII: S0957-4166(96)00243-1

Chemoenzymatic Route to ß-Blockers *via* 3-Hydroxy Esters

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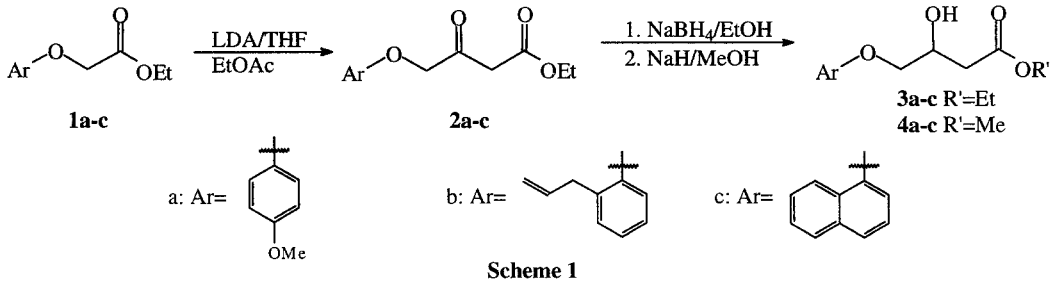
Abstract: Enantiomerically pure precursors of ß-blockers (propranolol, alprenolol and 1-(isopropylamino)-3-*p*-methoxy-phenoxy-2-propanol) were synthesized. Key step is the lipase-catalyzed kinetic resolution of *rac*-3-hydroxy esters either by O-acylation using vinyl acetate or by hydrolysis of the ester group. Both approaches were highly enantioselective (> 95 %ee) with E-values > 150 using lipase from *Pseudomonas cepacia.* The formal synthesis of (-)-(S)-propranolol was developed in subsequent steps.

INTRODUCTION

ß-Blockers are among the top twenty drugs based on their world wide sales. For example, the sales of atenolol valued more than 1 million US$ in 1990.[1] Although the (S)-enantiomers are much higher biologically active than the (R)-enantiomers and in some cases the (R)-enantiomer shows contraceptive actions, most ß-blockers are still sold as racemates. Both chemical and chemoenzymatic methods can yield enantiomerically pure ß-blockers. For example, Bevinakatti and Banerji resolved 1-chloro-3-(aryloxy)-2-propanol derivatives by lipase-catalysed hydrolysis or acylation for the preparation of (S)-propranolol, (S)-atenolol and (S)-practolol.[2] In another approach N-substituted acetates of propranolol were hydrolysed by several esterases, however only moderate enantioselectivities and a preference for the unwanted (R)-enantiomer were reported.[3] The preparation of (S)-propranolol via lipase-catalyzed resolution of glycerol derivatives[4] and cyanohydrins[5] was also performed. 3-(Aryloxy)-1,2-propanediol derivatives were used to develop an improved active site model of lipase from *Pseudomonas* cepacia.[6] In an earlier study[7], we have used 3-hydroxy esters for the investigation of factors affecting the enantioselectivity in the kinetic resolution by lipase catalysis in organic solvents. It turned out, that according to Kazlauskas' rule[8] for secondary alcohols, 3-hydroxy esters with a large aromatic group (namely 4-(1-naphthyloxy)-3-hydroxybutyric acid methyl ester) have been much better substrates and high enantioselectivities were observed.[7] In the present report, these findings were extended to the lipase-catalyzed resolution of other 4-(1-aryloxy)-3-hydroxybutyric acid esters. These can be transformed into the corresponding ß-blockers as has been elaborated in this paper for propranolol.

RESULTS

The 3-hydroxy ethyl esters **3a-c** were synthesized from the 2-aryloxy-acetic acid ethyl esters **1a-c** (Scheme 1) in two steps in about 80 % overall yield. It turned out, that methyl esters were better substrates and therefore we also performed later ester exchange with NaH in methanol to yield **4a-c**.



Scheme 1

Screening of suitable lipases

Typical results of the screening using commercial lipases are shown in Table 1 for the hydrolysis of the ethyl ester of **3c**. In all cases the product **5c** had the S-configuration (Scheme 3). This was confirmed by chiral GC analysis, chiral shift NMR spectroscopy and chemical correlation (see experimental). It is obvious from Table 1, that best results were achieved with lipase from *Pseudomonas cepacia* (PCL), giving an E-value of more than 150, which should allow the isolation of enantiomerically pure substrate and product. Another good lipase was from *Chromobacterium viscosum* (CVL). This may be due to the very high sequence homology to the *Pseudomonas sp.* lipase family.[9] PCL was used throughout all subsequent experiments.

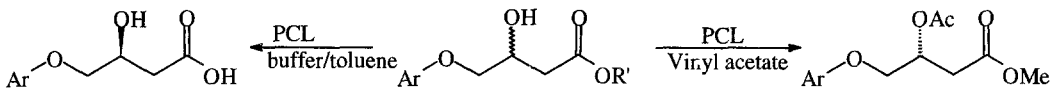
Table 1: Screening of lipases *via* hydrolysis of **3c**

Lipase	%ee **5c** (S)	%ee **3c** (R)	Time (h)	c* (%)	E*
PPL	92	96	69	35	15
PCL	90	96	22	48	>150
CRL	10	22	137	32	3
CVL	78	93	69	46	79
RML	9	13	137	55	< 2

*calculated from the enantiomeric excess according to Chen *et al.*[10]

*Resolution of the 3-hydroxy methyl esters **4a-c***

Compounds **4a-c** were subjected to either lipase catalyzed hydrolysis in aqueous phosphate buffer / toluene or acylation with vinyl acetate in hexane using PCL (Scheme 2). The hydrolysis of the three 3-hydroxy methyl esters **4a-c** resulted in all cases in the formation of highly pure acid in the desired (S)-configuration. The enantioselectivity was high in all cases and the reactions virtually stopped around 50 % conversion. The fasted reaction was observed with compound **4a**. On the other hand we found that the acylation with vinyl acetate was even more enantioselective and even faster, yielding the acetate in (R)-configuration. In the case of compounds **4a** and **4b** the reaction progress could be monitored by gas chromatography (GC) on a chiral column thus allowing the almost exact determination of 50 % conversion.



5a-c **3c/4a-c** **6a-c**

Scheme 2

Table 2: *Pseudomonas cepacia* lipase (PCL) catalyzed resolution of the 3-hydroxy esters **4a-c**

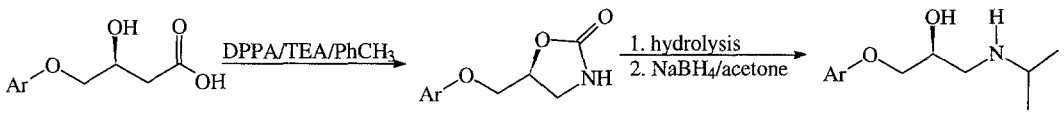
Method	$\%ee_s$	$\%ee_p$	Time (h)	c* (%)	E*
Hydrolysis	**4a** 97 (R)	**5a** 99 (S)	13	49	> 150
	4b 98 (R)	**5b** 98 (S)	78	49	> 150
	4c 98 (R)	**5c** 98 (S)	85	49#	> 150
Acylation	**4a** 99 (S)	**6a** 99 (R)	4	49	> 150
	4b 99 (S)	**6b** 99 (R)	8	49	> 150
	4c 49 (S)	**6c** 99 (R)	28	33#	> 150

#The separation of the enantiomers of **4c** was not possible by GC and therefore the reaction was stopped, when the conversion was estimated from TLC analysis to be close to 50 %.

*calculated from the enantiomeric excess according to Chen *et al.*[10]

Formal synthesis of (-)-(S)-propranolol

The preparation of (-)-(S)-propranolol is possible in a subsequent synthesis as depicted in Scheme 3. The oxazolidinone (S)-**7c** was obtained in good chemical yields (81 %) from the enantiomerically pure acid (S)-**5c**. All spectroscopical data were in accordance with literature values[11]. The subsequent conversion to (-)-(S)-Propranolol **8c** has already been described by Cardillo *et al.*[11] and was not repeated.



(S)-**5c** (S)-**7c** (S)-**8c** (Propranolol)

Scheme 3

Experimental

General methods: The lipases were from the following sources: PPL: Porcine pancreas lipase, Miles Kali-Chemie (Hannover, Germany); PCL: *Pseudomonas cepacia*, Amano P (Nagoya, Japan); CRL: *Candida rugosa* (formerly classified as *Candida cylindracea*), Amano AY 30; CVL: *Chromobacterium viscosum*, Toyo-Jozo Co (LP-215-S, Tokyo, Japan); RML: *Rhizomucor miehei*, Gist Brocades E (Delft, Netherlands).

Gas chromatographic analysis (HRGC Mega 2 series, Autosampler A 200S, software package Chrom Card for Windows, Fisons Instruments, Mainz-Kastel, Germany) was performed using a chiral stationary phase (hydrodex β-3P = heptakis-(2,3,6-tri-O-methyl)-γ-cyclodextrin, 25 m, i.d. 0.25 mm, Macherey & Nagel, Düren, Germany) and a flame ionization detector (FID). Conversion and enantioselectivity were calculated from the enantiomeric excess of substrate and product according to Chen *et al.*[10] NMR spectra were recorded on a Bruker WP200 (200 MHz) and AM 400 (400 MHz) in $CDCl_3$. IR-spectra were measured on a Bruker IFS 25, mass spectra using MAT 312 from Finnigan. Optical rotations were determined with a polarimeter 241

from Perkin-Elmer. The enantiomeric excess was verified by ^{1}H-NMR spectroscopy in the presence of (+)-Eu(hfc)$_3$ in $CDCl_3$.

Chemical synthesis: Compounds **1a-4c** were synthesized under nitrogen atmosphere in predried glass ware. Spectroscopical and analytical data of all compounds were correct. The literature known aryloxy acetic acid ethyl ester **1a-c** were synthesized here *via* their corresponding phenolates (from NaH in dimethylformamide) and bromoacetic acid ethyl ester followed by silica gel chromatography in approx. 90 % yields. Work-up was performed after hydrolysis -if not described otherwise - through addition of water, three times extraction with diethyl ether, washing of the combined organic layers with water, drying over magnesium sulfate and evaporation *in vacuo*. The raw products were purified by silica gel chromatography with diethyl ether/petrol ether mixtures. The reactions were monitored by thin layer chromatography (TLC).

3-Hydroxy-4-(4-methoxy phenoxy)-butanoic acid methyl ester **4a**: Diisopropylamine (12.1 g, 120 mmol) was dissolved in THF (100 ml). Butyllithium (75 ml, 120 mmol) was added dropwise at - 40 °C. The mixture was stirred for 30 min followed by cooling to - 50 °C and dry acetic acid ethyl ester (10.6 g, 120 mmol) in THF (25 ml) was added dropwise. After 1 hr stirring at - 70 °C 2-(4-methoxyphenoxy)-ethanoic acid ethyl ester **1a** (10.5 g, 50 mmol) in THF (25 ml) was added dropwise. After warming up to - 30 °C, the mixture was hydrolysed with 130 ml 2 N sulfuric acid and worked-up. The 4-(4-methoxyphenoxy)-3-oxo-butanoic acid ethyl ester **2a** (13.5 g) thus produced was reduced without further purification in dry ethanol (150 ml) under ice cooling for 1 hr through portion-wise addition of $NaBH_4$ (1.5 g, 40 mmol). After the reduction was terminated (3 hrs), the solution was acidified carefully with 2 N sulfuric acid and worked-up. Purification by silica gel chromatography gave 10.0 g (79 %) **3a**. Interesterification was performed by the addition of catalytical amounts of NaH to a solution of the ethyl ester **3a** (5.1 g, 20 mmol) in 100 ml methanol. After standing for a few hours at RT a few droplets of acetic acid were added, excess solvent was removed *in vacuo* and the methyl ester was purified by silica gel chromatography yielding 4.6 g (96 %) **4a**, mp. 81° C. ^{1}H-NMR ($CDCl_3$): δ = 2.67 (m, CH_2); 3.11 (s, OH); 3.73 (s, OCH_3); 3.76 (s, OCH_3); 3.95 (d, J = 6 Hz; CH_2O); 4.41 (m, CHO); 6.84 (s, 4-aryl-H) ppm. ^{13}C-NMR ($CDCl_3$): δ = 37.9; 51.88; 55.68; 66.79; 71.50; 114.67; 115.55; 152.58; 154.13; 172.47 ppm. IR ($CHCl_3$): ν = 3584, 3000, 2952, 2932, 2872, 2836, 1732, 1508, 1460, 1440, 1236, 1176, 1104, 1084, 1040 cm^{-1}. MS: m/e = 240 (M^+, 60), 222 (19), 209 (12), 191 (38), 167 (21), 166 (66), 149 (17), 147 (20), 138 (15), 137 (56), 131 (13), 124 (82), 123 (83), 118 (36), 117 (100), 110 (74), 107 (60), 99 (40), 95 (62), 92 (63), 85 (68), 77 (67). Enzymatic hydrolysis of *rac*-**4a** gave the corresponding acid (S)-**5a** (99 %ee): $[\alpha]_D^{20}$= - 9.48 (c=0.795, $CHCl_3$), mp. 81-82°C (CH_2Cl_2 / PE); acylation the acetate (R)-**6a** (97 %ee): $[\alpha]_D^{20}$= + 25.00 (c=1.255, $CHCl_3$), oil, and (S)-**4a** (97 %ee): $[\alpha]_D^{20}$= - 3.30 (c=0.93, MeOH), mp. 81°C.

3-Hydroxy-4-(2-prop-2-enylphenoxy)-butanoic acid methyl ester **4b**: Synthesis was performed as described for **4a**. Starting from 2-(prop-2-enylphenoxy)-ethanoic acid ethyl ester **1b** (11.0 g, 50 mmol), 15.0 g 3-Oxo-4-(2-prop-2-enylphenoxy)-butanoic acid ethyl ester **2b** was isolated, which was reduced to the 3-hydroxy-4-(2-prop-2-enylphenoxy)-butanoic acid ethyl ester **3b** (11.0 g, 83 %, colorless oil). The interesterification of **3b** (5.0 g, 19 mmol) with methanol in the presence of NaH gave 4.8 g (100 %) **4b** as colorless oil. ^{1}H-NMR ($CDCl_3$): δ = 2.70 (m; CH_2); 3.06 (s; OH); 3.39 (dt, J = 7 Hz, J = 2 Hz; CH_2); 3.73 (s; CH_3); 4.00 (m; CH_2O); 4.44 (m; CH_2); 5.01 (dt, J = 6 Hz, J = 2 Hz; CH); 5.08 (t, J = 2 Hz; CH); 6.0 (m; CH); 6.8-7.0 and 7.1-7.3 (2m, 4 aryl-H) ppm. ^{13}C-NMR ($CDCl_3$): δ = 34.64; 38.05; 31.89; 66.82; 70.72; 111.31; 115.34; 121.11; 127.51; 128.47; 130.13; 137.21; 156.07; 172.37 ppm. IR (cap. film): ν = 3464, 3076, 3024, 3004, 2976, 2952, 2928, 1736,

1636, 1600, 1492, 1452, 1436, 1244, 1172, 1112, 1088, 1048 cm^{-1}. MS: m/e = 250 (M^+, 36), 232 (6), 219 (9), 201 (10), 176 (20), 159 (33), 157 (11), 147 (23), 145 (19), 143 (36), 141 (20), 131 (44), 119 (41), 117 (100), 115 (41), 107 (37), 91 (45), 85 (52), 77 (39). Enzymatic hydrolysis of *rac*-**4b** gave the corresponding acid (S)-**5b** (98 %ee): $[\alpha]_D^{20}$= - 8.72 (c=1.560, MeOH), oil; acylation the acetate (R)-**6b** (98 %ee): $[\alpha]_D^{20}$= + 30.58 (c=1.615, $CHCl_3$), oil, and (S)-**4b** (98 %ee): $[\alpha]_D^{20}$= - 10.63 (c=1.355, MeOH), oil.

3-Hydroxy-4-(naphthalen-1-yloxy)-butanoic acid methyl ester **4c**: 2-(naphthalen-1-yloxy)-ethanoic acid ethyl ester **1c** (11.5 g, 50 mmol) yields analog to the synthesis of **2a**, 13.7 g raw 4-(naphthalen-1-yloxy)-3-oxo-butanoic acid ethyl ester **2c**, which was reduced to the 3-hydroxy-4-(-naphthalen-1yloxy)-butanoic acid ethyl ester **3c** (11.7 g, 85 %, colorless oil). ^{1}H-NMR ($CDCl_3$): δ = 1.20 (t, J = 7 Hz; CH_3), 2.70 (m; CH_2); 3.60 (s; OH); 4.10 (m; CH_2O); 4.12 (q, J = 7 Hz; CH_2); 4.53 (m; CHO); 6.71 (dd, J = 7.5 Hz, J = 1 Hz; 1 aryl-H); 7.18-7.48 (m; 4 aryl-H); 7.74 (m; 1 aryl-H); 8.23 (m; 1 aryl-H) ppm. ^{13}C-NMR ($CDCl_3$): δ = 14.09; 38.43; 60.85; 66.81; 70.96; 104.92; 120.69; 121.84; 125.25; 125.48; 125.77; 126.43; 127.48; 134.47; 154.08; 172.15 ppm. IR (cap. film): ν = 3450, 2955, 1730, 1580, 1510, 1460, 1400, 1270, 1105, 795, 770 cm^{-1}. MS: m/e = 274 (M^+, 23), 229 (12), 211 (6), 183 (8), 157 (8), 144 (75) 131 (100), 115 (41), 103 (80), 96 (2), 85 (20), 76 (9).

The interesterification of **3c** (5.7 g, 21 mmol) with methanol in the presence of NaH gave 5.2 g **4c** (90 %); mp. 61-62 °C. ^{1}H-NMR ($CDCl_3$): δ = 2.75 and 2.83 (dd; J = 16 Hz, J = 7 Hz and dd, J = 16 Hz, J = 5 Hz; CH_2); 3.21 (s; OH); 3.85 (s; OCH_3); 4.16 and 4.22 (dd, J = 10 Hz, J = 6 Hz and dd, J = 9 Hz, J = 6 Hz; CH_2); 4.58 (m; CH); 6.82 (dd, J = 8 Hz, J = 1 Hz; 1 aryl-H); 7.30-7.58 (m; 4 aryl-H); 7.79 (m; 1 aryl-H); 8.23 (m; 1 aryl-H) ppm. ^{13}C-NMR ($CDCl_3$): δ = 38.15; 51.90; 66.82; 70.90; 104.92; 120.78; 121.77; 125.30; 125.46; 125.76; 126.47; 127.52; 134.48; 154.03; 172.55 ppm. IR ($CHCl_3$): ν = 3592, 3056, 3000, 2956, 2936, 1732, 1580, 1508, 1460, 1440, 1404, 1268, 1240, 1104, 1068, 1020, cm^{-1}. MS: m/e = 260 (M^+, 51), 242 (10), 229 (22), 211 (27), 187 (25), 186 (51), 169 (19), 158 (30), 157 (48), 155 (11), 144 (81), 127 (58), 117 (100), 115 (73), 101 (29), 99 (49), 90 (40), 85 (57), 77 (46). Enzymatic acylation of *rac*-**4c** gave the corresponding acetate (R)-**6c** (99 %ee): $[\alpha]_D^{20}$= + 27.96 (c=1.08, $CHCl_3$), oil, and (S)-**4c** (99 %ee): $[\alpha]_D^{20}$= - 4.91 (c=1.16, MeOH), oil.

*(+)-(S)-5-(Naphthalen-1-yloxymethyl)-oxazolidin-2-on, (+)-(S)-***7**[11]*:* (-)-(S)-**5c** was obtained through lipase-catalyzed hydrolysis in phosphate buffer (pH 7) in 46 % yield (2.7 g, 98 % ee, $[\alpha]_D^{20}$= -15.76 (c=1.225, $CHCl_3$), mp. 69 °C). ^{1}H-NMR ($CDCl_3$): δ = 2.74 (d, J = 6 Hz; CH_2); 4.03 (d, J = 6 Hz; CH_2O); 4.5 (quint., J = 6 Hz; CHO); 6.60 (d, J = 8 Hz; 1 aryl-H); 7.15-7.45 (m, 4 aryl-H, 2-OH); 7.75 (m, 1 aryl-H); 8.22 (m; 1 aryl-H) ppm. ^{13}C-NMR ($CDCl_3$): δ = 38.14; 66.83; 70.61; 104.92; 120.82; 121.74; 125.34; 125.38; 125.74; 128.84; 127.50; 134.44; 153.88; 176.75 ppm. IR (cap. film): ν = 3450, 2955, 1730, 1580, 1510, 1460, 1400, 1270, 1105, 795, 770 cm^{-1}.

1.23 g (5 mmol) of (-)-(S)-**5c** were heated with 1.25 g diphenylphosphorylazide (DPPA[12], 6 mmol) and 0.61 g triethylamine (6 mmol) in 60 ml dry toluene for 4 hrs to 80-90°C. After evaporation *in vacuo* the mixture was dissolved in methylene chloride and worked-up by addition of hydrogen carbonate solution and water. After silica gel chromatography (ether:methanol = 95:5) and crystallization from chloroform/petrol ether, 1.08 g (89 %) (+)-(S)-**7** was isolated, mp.: 155 °C, $[\alpha]_D^{20}$= +12.8 (c=0.70, ethanol); lit.: mp.: 149 °C, $[\alpha]_D^{20}$= +12.2 (c=0.80, ethanol). All spectroscopical data are in accordance with literature values.

Assignment of absolute configurations: The absolute configurations of the products (R)-**4c** and (S)-**5c**, (S)-**4c** and (R)-**6c** of the 1-naphtyloxy derivatives were determined *via* transformation of (-)-(S)-**5c** into the literature known (+)-(S)-**7c**. The tentatively assignment of the configuration of the analogous products (R-)-**4a**, (S)-**5a,** (S)-**4a** and (R)-**6a** of the 4-methoxyphenoxy series and the products (R)-**4b**, (S)-**5b,** (S)-**4b** and (R)-**6b** of the

2-allylphenoxy series was assumed from the similar behavior observed in the elution order in chiral gas chromatography on a chiral column and the relative position of the residences in NMR spectroscopy in the presence of (+)-Eu(hfc)$_3$.

Lipase catalyzed reactions: Transesterifications were carried out at 37°C in 10 ml glass stoppered round bottom flasks in an oil bath. The magnetic stirrer speed was kept at 400 rpm. In a typical experiment 50 mg (0.3 mmol) (R,S)-**4a**, 50 µl (0.3 mmol) vinyl acetate, 50 mg PCL and 2 ml hexane were added. Samples withdrawn during the reaction were centrifugated to separate the enzyme. The supernatant was derivatized with trifluoroacetic acid anhydride as described previously[7] and analyzed by GC. Hydrolysis was carried out in a biphasic reaction system consisting of 50 mM phosphate buffer (pH 7) : toluene (1:1) at 37°C in a pH-stat system (Metrohm, Buchs, Switzerland). After consumption of 0.1 M NaOH indicated approximately 50 % conversion, the reaction was stopped. After separation of substrate and product, the acid was converted into the methyl ester by addition of etheral diazomethane solution and analyzed by GC.

Acknowledgements

The authors are grateful to Prof. R. D. Schmid (University of Stuttgart, Stuttgart, Germany) for his kind support and useful discussion and the companies listed in materials and methods for their generous gifts of lipase samples.

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(*Received in UK* 24 *April* 1996)