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Stereoselective Acetylation of 3,4-Dihydro-3,4-Dihydroxy-2,2-Dimethyl-2H-1- Benzopyran-6-Carbonitrile

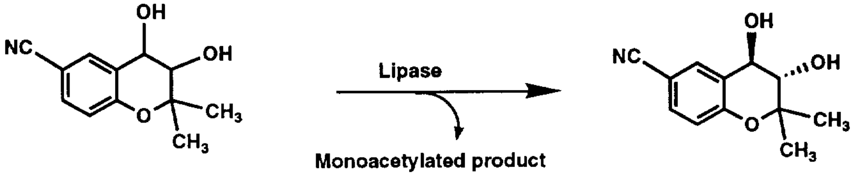
Ramesh N. Patel, Amit Banerjee, Clyde G. McNamee, and Laszlo J. Szarka

Department of Microbial Technology, Bristol-Myers Squibb Pharmaceutical Research
Institute, P.O. Box 191, New Brunswick, N. J. 08903

ABSTRACT. The chiral intermediate diol (3*S*,4*R*)-trans-3,4-dihydro-3,4-dihydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile 2, was prepared for the total synthesis of a potassium channel opener drug candidate. The stereoselective acetylation of racemic 1 was carried out with various lipases among which the lipases from Candida cylindraceae and Pseudomonas cepacia catalyzed the acetylation of the undesired enantiomer of racemic 1 to yield monoacetylated product and unreacted desired (+)-trans diol 2. A reaction yield of >40% and an optical purity >90% were obtained using each lipase.

INTRODUCTION

The current interest in enzymatic production of chiral diols lies in the preparation of intermediates for chemical synthesis.[1-6] The study of potassium (K) channel biochemistry, physiology, and medicinal chemistry has fluorished, and numerous papers and reviews have been published in recent years.[7-10] It has long been known that K channels play an important role in neuronal excitability [10], and it is now clear that K channels play a complex and critical role in the basic electrical and mechanical functions of a wide variety of tissues, including smooth muscle, cardiac muscle and glands.[11] A new class of higly specific pharmacological compounds has been developed which open or block K channels.[9-14] K channel openers are powerful smooth muscle relaxants with in vivo hypotensive and bronchodilator activity typically demonstrated by compounds like cromakalim, nicorandil, and pinacidil.[11] Recently, the synthesis and antihypertensive activity of a series of novel K channel openers[14-17] based on monosubstituted trans-4-amino-3,4-dihydro-2,2-dimethyl-2H-1-benzopyran-3-ol have been demonstrated. In this report we describe the stereoselective enzymatic acetylation of a racemic diol, 4-dihydro-3,4-dihydroxy-2,2-dimethyl-2H-1-benzopyran-6-carbonitrile 1, to yield the monoacetylated product and the unreacted desired (+)-trans diol 2 [(3*S*,4*R*)-trans-3,4-dihydro-3,4-dihydroxy-2,2-dimethyl-2H-1-benzopyran-6-carbonitrile] as shown in scheme 1.



Racemic Diol 1

(+)-Trans diol 2

RESULTS AND DISCUSSION

Commercially available lipases from *Candida cylindraceae* (Biocatalyst), *Rhizopus niveus* (lipase N, Amano International), *Penicillium* sp. (lipase F12, Enzymatics), *Aspergillus usamii* (lipase F10, Enzymatics), *Mucor* sp. (lipase MAP10, Amano International), and *Pseudomonas cepacia* (lipase PS-30, Amano International) were evaluated for acetylation of racemic 1 in an organic solvent (toluene) in the presence of isopropenyl acetate as acyl donor. LipasePS-30 from Pseudomonas cepacia, and Candida cylindraceae lipase effectively catalyzed the acetylation of the undesired enantiomer of racemic diol 1 to yield a monoacetylated product and unreacted diol enriched in the desired (+)-trans diol 2. A reaction yield of >42% and optical purity of >92% were obtained (Table 1). Other lipases had very low activity.

TABLE 1: Lipase screen for the stereoselective acetylation of racemic diol 1

Lipase source	Reaction yield (%)	Conversion (%)	Optical purity of (+)-trans diol 2 (%)
Candida cylindraceae	42	58	95
Pseudomonas cepacia Lipase PS-30	45	55	92

Reactions were carried out as described in the Experimental section. Thereaction yield and optical purity were determined by HPLC.

Preparative scale resolution of racemic **1** was carried out in a 1-L reactor using crude lipase from *Candida cylindraceae* in toluene as solvent. The kinetics of the resolution process are as shown in Table 2. A reaction yield of 43% and optical purity of 96% were obtained for unreacted desired (+)-trans diol **2**. From the reaction mixture, 3.8 g of (+)-trans diol **2** was isolated in 38% overall yield (maximum theoritical yield is 50%). An optical purity of 98% and a chemical purity of 98.5% were obtained for the isolated (+)-trans diol **2**.

TABLE 2. Kinetics of acetylation of racemic diol by *Candida cylindraceae* lipase

Reaction time (hours)	Diol (g/L)	Monoacetylated Product (g/L)	Optical purity of (+)-trans diol **2** (%)
0	10	0	50
12	9.1	1.2	54
24	7.9	1.94	61
36	6.8	3.4	72
48	6.1	3.95	82
60	5.45	4.8	91
72	4.6	5.6	94
80	4.35	5.9	96

The reaction mixture contained 920 mL of toluene, 80 mL of isopropenyl acetate, 1 mL of water, 10 grams of racemic substrate **1** and 10 grams of crude *Candida cylindraceae* lipase. The reaction was carried out at 30°C, and 150 RPM. Substrate and product concentrations were determined by HPLC. Optical purities were determined by chiral HPLC.

Lipases have been used to catalyse esterification, transesterification, transthioesterification, and interesterification reactions in organic solvent. Both stereoselective and regioselective acylation reactions have been conducted to prepare intermediates for chemical synthesis. [18-26] The lipase-catalysed resolution reactions conducted by hydrolysis are shifted toward completion due to the high concentration of the nucleophile water. In contrast, the lipase-catalysed esterification and transesterification reactions are generally carried out in organic solvent in the presence of excess acyl donor and nucleophile alcohol, hence the reaction is not well controlled. The enantiomeric excess of both substrate and product will decrease when the extent of conversion is exceeded.[27] In the present studies, lipase from *Candida cylindraceae* and lipase PS-30 from *Pseudomonas cepacia* catalysed the acetylation of the undesired enantiomer of racemic **1**. Thus the enantiomeric excess of the desired (+)-trans diol **2** remained the same or increased upon greater than 50% conversion.

Hirtake et al. used vinyl acetate as an acyl donor for enzymatic transesterification reactions.[28] In such a reaction, the enol that is formed upon the reaction of the acyl donor with the alcohol substrate is tautomerized to acetaldehyde and the reaction becomes irreversible. Under these circumstances, the kinetics of the resolution process can be described as suggested by Chen *et al.*[27] Lipase-catalyzed transesterification alcohols, glycerol derivatives, and sugars using enol esters as the acyl donor has been described by Wang et al. 29,30. In this report, we have used isopropenyl acetate as an acyl donor. The advantage is the generation of acetone instead of acetaldehyde during lipase-catalysed acetylation. Acetone is much more compatible with the enzymatic process than acetaldehyde which is reactive and can cause inactivation of lipase at high concentration.

EXPERIMENTAL

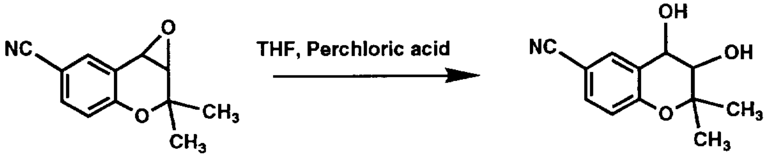
Racemic epoxide **3** and standard chiral epoxide **4** were synthesised by Chemical Process Research, Bristol-Myers Squibb Pharmaceutical Research Institute by literature procedures.[14-15] The proton magnetic resonance (^{1}H-NMR) and carbon magnetic resonance (^{13}C-NMR) were recorded on a Brucker AM-300 spectrometer.The physicochemical properties, including spectral characteristics (^{1}H-NMR, ^{13}C-NMR, Mass spectra), were in full accord for all these compounds.

(cis)-3,4-Dihydro-2,2-dimethyl-2H oxireno [c] [1] benzopyran-6-carbonitrile **3**:

Racemic epoxide was prepared as described earlier.[15] mp.142-144°C; NMR ($CDCl_3$) δ 1.15 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 3.35 (d, J= 4 Hz, 1H, H-3), 3.92 (d, J= 4 Hz, 1H, H-4), 6.84 (d, J=10 Hz, H-8), 7.28 (s, 1H, H-5), 7.42 (d, J= 6 Hz, H-7). 13C-NMR ($CDCl_3$), 75.46 MHz) δ 156.4, 134.3, 133.7, 121.0, 118.93, 118.67, 104.18, 74.6, 62.2, 49.76, 25.4, 22.9; Anal Calcd for $C_{12}H_{11}NO_2$: C, 71.56; H, 5.52; N, 6.95; Found: C, 71.46; H, 5.46; N, 7.04. Mass spectrum, m/z 201. (m^+).

(+/-)-Trans-3,4-dihydro-3,4-dihydroxy-2,2-dimethyl-2H-1-benzopyran-6-carbonitrile 1:

This compound was prepared from the corresponding racemic epoxide (cis)-3,4-Dihydro-2,2-dimethyl-2H oxireno [c] [1] benzopyran-6-carbonitrile 3. Racemic epoxide (40 gram, 0.2 mole) was dissolved in 1L of tetrahydrofuran (THF). To this solution 40 mL of water and 2 mL of perchloric acid were added. The reaction mixture was stirred at room temperature for 30 minutes. The progress of the reaction was followed by gas chromatography. After all epoxide was converted, 200 mL of water was added and the resulting reaction mixture was extracted twice with 250 mL of dichloromethane. The organic phase was washed with 0.7M sodium bicarbonate solution. The organic phase was then dried over anhydrous sodium carbonate. The solvent was evaporated under reduced pressure to produce 35 grams, 0.16 mole (80 % molar yield) of white waxy solid of 98% chemical purity as analyzed by GC.



Racemic epoxide 3 Racemic Diol 1

NMR ($CDCl_3$) δ 1.22 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 3.18 (s, 2H, OH), 3.62 (d, J= 8.5 Hz, 1H, H-3), 4.68 (d, J= 8.5 Hz, 1H, H-4), 6.82 (d, J= 10 Hz, 1H, H-8), 7.31 (s, 1H, H-5), 7.4 (d, J= 1 Hz, 1H, H-7). ^{13}C-NMR ($CDCl_3$), 75.46 MHz) δ 156.16, 133.14, 132.55, 124.7, 119.35, 117.97, 103.56, 75.46, 68.48, 49.45, 26.97, 26.61; Mass spectrum, m/z 219 (m+). Anal. Calcd. for $C_{12}H_{13}NO_3$: c, 65.71; H, 5.93; N, 6.38; Found: C, 65.82, H, 6.01; N, 6.45.

(3S, 4S-cis)-3,4-Dihydro-2,2-dimethyl-2H-oxireno [c] [1] benzopyran-6-carbonitrile 4:

Chiral epoxide (3S, 4S-cis)-3,4-Dihydro-2,2-dimethyl-2H-oxireno [c] [1] benzopyran-6-carbonitrile 4 was synthesized using Jacobson's catalysts. [15] $[\alpha]_D^{25}$= −89.3 (C=1.01, MeOH). Anal Calcd for $C_{12}H_{11}NO_2$: C, 71.56; H, 5.52; N, 6.95; Found: C-71.46; H, 5.46; N, 7.04. Mass spectrum, m/z 201. (m^+).

(3S, 4R)-Trans-3,4-dihydro-3,4-dihydroxy-2,2-dimethyl-2H-1-benzopyran-6-carbonitrile 2:

This compound was prepared from the corresponding chiral epoxide 4 utilizing the above procedure.Anal. Calcd. for $C_{12}H_{13}NO_3$: C, 65.71; H, 5.93; N, 6.38; Found: C, 65.75; H, 5.98; N, 6.42. $[\alpha]_D^{25}$ = + 16.2 (c- 1.02, MeOH).

Enzyme source

Crude lipase from *Candida cylindraceae* was purchased from the Biocatalyst (UK) . Lipases from *Rhizopus niveus* (lipase N), *Mucor* sp. (lipase MAP10), *Pseudomonas cepacia* (lipase PS-30) were obtained from Amano International (USA). Lipases from *Penicillium* sp. (lipase F12) and *Aspergillus usamii* (lipase F10) were purchased from Enzymatics (UK).

Enzymatic reaction

The enzymatic reaction mixture for lipase screen contained 40 mg of racemic diol **1**, 100 µL of isopropenyl acetate, 10 µL of water, 200 mg of crude lipase in 10 mL of toluene. The reaction was carried out in a teflon screw-capped flask at 28°C and 280 RPM on a rotary shaker for 48 hours. The reaction yield and optical purity of (+)-trans diol were determined by HPLC.

Preparative-scale resolution of racemic diol 1

The resolution of racemic diol **1** to the corresponding (+)-trans diol **1** by the acetylation reaction using *Candida cylindraceae* lipase was scaled-up to 1-L batch. The reaction mixture contained 920 mL of toluene, 80 mL (0.8 moles) of isopropenyl acetate, 1 mL of water, 10 grams (0.045 mole) of racemic substrate **1** and 10 grams of crude *Candida cylindraceae* lipase. The reaction was carried out at 30°C and 150 RPM. Substrate and product concentrations were determined by HPLC. Optical purities were determined by chiral HPLC. At the end of the reaction, the reaction mixture containing enzyme was filtered through a sintered glass filter to remove enzyme. The filtrate (toluene) containing product was extracted twice with 2 L of water . The aqueous layer containing (+)-trans diol **1** was collected, 20 grams of SP-207 resin was added, and the suspension was stirred overnight. The resin-bound chiral diol **1** was collected by filtration through a sintered glass filter and dried under vacuum at room temperature. The dried resin was washed with 100 mL of hexane and extracted twice with 100 mL of tert-butyl methyl ether. The organic layer containing (+)-trans diol **1** was dried over anhydrous sodium sulfate and then concentrated under reduced pressure to obtain 4.8 grams (0.022 moles) of yellow waxy solid. (+)-trans diol **1** was further purified by preparative column chromarography using a C-18 column and methanol:water (1:1) as the mobile phase to obtain 3.8 grams (0.017 moles) of (+)-trans diol **1** in 38% overall yield (maximum theoretical yield is 50%).

Isolation of Monoacetylated Product

The water washed toluene (0.9 L) from the enzymatic reaction mixture was concentrated under reduced pressure, to obtain a light yellow waxy solid (5 g). The solid was washed with 50 mL of cyclohexane, then dissolve in 25 mL of dichloromethane. Chromatography of this material on a silica gel column (100 g) with hexane:ether (80:20) gave 3.5 g of white solid (0.013 moles) of the monoacetate (yield 29%, maximum theoritical yield is 50%) . The chemical purity of the compound was 97% as analysed by GC.

^{1}H-NMR, 300 MHz, ($CDCl_3$) δ 1.22 (s, 3 H, CH_3), 1.45 (s, 3 H, CH_3), 2.07 (s, 3 H, acetyl), 3.20 (s, 1 H, OH), 3.68 (d, J = 8.5 Hz, 1 H, H-3), 4.95 (d, J = 8.8 Hz, 1 H, H-4), 6.84 (d, J = 10 Hz, 1H, H-8), 7.31 (s, 1 H, H-5), 7.4 (d, J =10 Hz, 1 H, H-7). ^{13}C-NMR ($CDCl_3$), 75.46 MHz,

δ 170.28, 150.5, 155.1, 133.41, 133.17, 133.04, 118.01, 77.04, 66.84, 29.59, 22.85, 20.86, 19.57. Mass spectrum m/z, 262, (m^+).

Analytical methods

Analysis of racemic compounds 1, and monoacetylated product was carried out by using a HewlettPackard 5890 gas chromatograph with a flame ionization detector (FID). An HP-2 fused-silica capillary column (25 m long x 0.32 mm Int. diameter x 0.17 mm thickness) at 250°C injection temperature, 250°C detector temperature and 195°C oven temperature was used. The carrier gas was helium and total run time was 8 min. The retention time for diol 1 and monoacetytlated product were 6.53 min and 6.94 min respectively. The optical purity of (+)-trans diol 1 was determined by chiral HPLC. Reaction samples (1 mL) were dried under a gentle stream of nitrogen. The residue was suspended in 2 mL of hexane and passed through a silica cartridge (Analytichem Bond Elut LRC) previously equilibrated with hexane. The column was washed with 10 mL hexane and compound 3 was eluted with 10 mL of 50% acetone in hexane. All solvent was evaporated under a gentle stream of nitrogen and the residue was dissolved in 1 mL of 50% isopropanol in hexane. Samples were filtered through a 0.4 mm filter and analyzed by HPLC. The column was a Chiralcel OD (250 x 4.6 mm, Diacel Chemical Industries). The mobile phase consisted of 95:4:1 of hexane:n-butanol:cyclohexanol. The flow rate was 0.8 mL/min and the detector wavelength was 254 nm. The retention times for the two stereoisomers of trans diols were 29.4 min and 33.2 min respectively.

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