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Stereoselective acetylation of racemic 7-[*N*,*N*′-bis-(benzyloxycarbonyl)-*N*-(guanidinoheptanoyl)]-α-hydroxyglycine

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Abstract: Chiral intermediate (−)-7-[*N*,*N*′-Bis(benzyloxycarbonyl)-*N*-(guanidinoheptanoyl)]-α-acetoxyglycine **2** was prepared for the total synthesis of a (−)-15 deoxyspergualin, an antitumor antibiotic and immunosuppressive agent. The stereoselective acetylation of racemic 7-[*N*,*N*′-Bis(benzyloxycarbonyl)-*N*-(guanidinoheptanoyl)]-α-hydroxyglycine **1** was carried out in methyl ethyl ketone (MEK) using lipase from *Pseudomonas* sp. (lipase AK). Vinyl acetate was used as an acylating agent. A reaction yield of 48% (theoretical max 50%) and an optical purity of 98% were obtained for S-(−)-acetate **2**. The unreacted alcohol (+)-**1** was obtained in 41% yield and 98.5% optical purity. © 1997 Elsevier Science Ltd

Introduction

The current interest in enzymatic production of chiral compounds lies in the preparation of intermediates for pharmaceutical synthesis.[1–8] An antitumor antibiotic spergualin was discovered in the culture filtrate of a bacterial strain BMG162-aF2 which is related to *Bacillus laterosporus*, and its structure was determined to be (−)-(15S)-1-amino-10-guanidino-11,15-dihydroxy-4,9,12-triazanonadecane-10,13-dione.[9,10] The total synthesis was accomplished by the acid-catalyzed condensation of 11-amino-1,1-dihydroxy-3,8-diazaundecane-2-one with (S)-7-guanidino-3-hydroxyheptanamide followed by the separation of the 11-epimeric mixture.[11] Antibacterial or antitumor activity of the enantiomeric mixture of spergualin was about half of that of the natural spergualin[12] indicating the importance of the configuration at C-11 for antitumor activity.

Umeda et al.[13] has demonstrated the optical resolution of the key intermediate of 15-deoxyspergualin, racemic *N*-(7-guanidino-heptanoyl)-α-alkoxyglycine, by use of an exopeptidase (serine carboxypeptidase) and racemic *N*-(7-guanidino-heptanoyl)-α-alkoxyglycyl-L-amino acid as the substrate. Carboxypeptidase from *Penicillum janthinellum* catalyzed the hydrolysis of peptide bond of racemic *N*-(7-guanidinoheptanoyl)-α-methoxyglycyl-L-phenylalanine to yield (−)-*N*-(7-guanidinoheptanoyl)-α-methoxyglycine. They deduced that the absolute configuration of the carbon at 11 (C-11) of the bioactive (−)-enantiomer, and so that of the natural spergualin, is *S*. The (−)-enantiomer of 15-deoxyspergualin was active against mouse leukemic L1210, while the (+)-enantiomer was almost inactive.[13]

In this report we have demonstrated an alternate and more direct route, the lipase-catalyzed stereoselective acetylation of racemic 7-[*N*,*N*′-bis(benzyloxycarbonyl)-*N*-(guanidinoheptanoyl)]-α-hydroxy-glycine **1** to the corresponding S-(−)-acetate **2** and unreacted alcohol (+)-**1**. S-(−)-acetate **2** is a key intermediate for the total chemical synthesis of (−)-15-deoxyspergualin **3**, a related immunosuppressive agent and antitumor antibiotic.[12–14]

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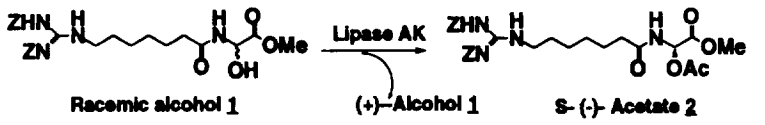
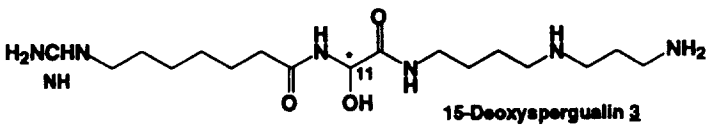
Table 1. Evaluation of lipases for the resolution of racemic **1**

Enzyme	Reaction yield of **1** (%)	Optical purity of **1** (%)	Reaction Yield of **2** (%)	Optical purity of **2** (%)
Lipase PS-30	90	53	8	ND
Lipase AK	41	98.8	46	98
Lipase OF	19	45	75	57
Esterase 30000	92	48	7	ND
PPL	93	48	6	ND

Reaction mixture in 10 mL of toluene contained 20 mg of substrate **1**, 200 mg of crude lipase as indicated, 0.8 mL of isopropenyl acetate, and 0.1% water. Reactions were carried out at 28°C, 200 RPM on a rotary shaker. The reaction yields and optical purities were determined by HPLC. ND= not determined.





Results and discussion

Commercially available lipases [lipase PS-30, lipase AY-30, lipase PG, lipase AK, lipase OF, lipase R, lipase MAP-10, lipase GC-20, Porcine pancreatic lipase (PPL), and esterase 3000] were screened for the stereoselective acetylation of racemic **1** in an organic solvent (toluene) in the presence of isopropenyl acetate (IPAC) as acyl donor. Lipase AY-30, lipase PG, lipase R, PPL, and lipase GC-20 were inactive in the enzymatic resolution process. Lipase AK from *Pseudomonas* sp. efficiently catalyzed the enantioselective acetylation of the desired enantiomer of racemic **1**. A reaction yield of 46% and an optical purity of 98% were obtained for S-(−)-acetate **2**. A reaction yield of 41% and an optical purity of 98.8% were obtained for the unreacted (+)-alcohol **1** (Table 1).

Since substrate was soluble in methyl ethyl ketone (MEK), we evaluated MEK and toluene as solvent with isopropenyl acetate and vinyl acetate as acyl donor in the enzymatic reaction using lipase AK. Results are as shown in the Table 2. MEK and vinyl acetate were found to be best combination for the acetylation of **1**. A reaction yield of 48% and an optical purity of 98.4% were obtained for S-(−)-acetate **2**. Chiral (+)-alcohol **1** was obtained in 40% reaction yield and 98.8% optical purity.

Enzymatic acetylation of **1** by lipase AK was scaled-up to a 2-L batch as described in the Experimental section. After a 10-day reaction period, a reaction yield of 48% and an optical purity of 98% were obtained for S-(−)-acetate **2**. Chiral (+)-alcohol **1** was obtained in 44% yield with 98% optical purity. From the reaction mixture, 1.5 g of S-(−)-acetate **2** was isolated in overall 37% yield (theoretical max. yield is 50%). An optical purity of 98% and a chemical purity of 98% were obtained for the isolated S-(−)-acetate **2**. Specific rotation $[\alpha]_D^{25}$ of −6.2 (C=1 in CH_2Cl_2) was obtained for S-(−)-acetate **2**. From the same reaction mixture, 0.98 g of (+)-alcohol **1** was isolated in a 98% chemical purity and a 98.9% optical purity.

The lipase-catalyzed resolution reactions conducted by hydrolysis are shifted toward completion due to the high concentration of nucleophile water. In contrast, the lipase-catalyzed esterification and transesterification reactions are generally carried out in organic solvent in the presence of excess acyl

Table 2. Enzymatic resolution of racemic **1** using lipase AK

Solvent	Acyl Donor	Reaction yield of 1 (%)	Optical purity of 1 (%)	Reaction yield of 2 (%)	Optical purity of 2 (%)
MEK	Vinyl acetate	40	98.8	48	98.4
MEK	IPAC	34	96.7	55	94
Toluene	Vinyl acetate	32	96	57	92
Toluene	IPAC	40	97	44	97.7

Reaction mixture in 10 mL of solvent contained 20 mg of substrate 1, 200 mg of crude lipase, 0.8 mL of acyl donor as indicated, and 0.1% water. Reactions were carried out at 28°C, 200 RPM on a rotary shaker. The reaction yields and optical purities were determined by HPLC. ND= not determined.

donor and nucleophile alcohol, hence the reaction is not well controlled. The enantiomeric excess of product will decrease when the extent of conversion is exceeded.[15] Hirtake et al. used vinyl acetate as an acyl donor for enzymatic transesterification reaction.[16] 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 circumtances, the kinetics of the resolution process can be described as suggested by Chen et al.[15] Lipase-catalyzed transesterification of alcohols, glycerol derivatives, and sugars using isopropenyl acetate as the acyl donor has been described by Wang et al.[17] The advantage is the generation of acetone instead of acetaldehyde during lipase-catalyzed acylation. Acetone is more compatible with the enzymatic process than acetaldehyde which is more reactive and can cause inactivation of lipase at high concentration.

In this report we have demonstrated an alternate route, the lipase-catalyzed synthesis of chiral S-(−)-acetate **2**, an intermediate for the total chemical synthesis of (−)-15-deoxyspergualin **3**.

Experimental

Materials

Starting substrate **1** and reference compounds racemic acetate **1**, single enantiomer of **1** and single enantiomer of **2**, were synthesized by colleagues in the Chemical Process Research Department, Bristol-Myers Squibb Pharmaceutical Research Institute as described previously[12]. The physico-chemical properties including spectral characteristics (^{1}H-NMR, ^{13}C-NMR, Mass spectra) were in full accord for all these compounds. The proton magnetic resonance (^{1}H-NMR) and carbon magnetic resonance (^{13}C-NMR) were recorded on a Brucker AM-300 spectrometer.

Enzyme source

Crude lipases from *Pseudomonas cepacia* (lipase PS-30), *Pseudomonas* sp. (lipase AK) *Rhizopus* sp. (lipase R), *Mucor* sp. (lipase MAP-10), *Candida cylindraceae* (lipase AY-30) were purchased from Amano International (USA). Porcine pancreatic lipase was purchased from Sigma Chemicals (USA). Lipase OF was purchased from Sepracor (USA) and Esterase 30,000 was purchased from Gist/Brocarde (France).

Acetylation of 1 by lipase

Typically, the reaction was carried out in 9.9 mL of toluene or MEK (dried over 4 Å molecular sieve) using 2 mg/mL of substrate **1**, 20 mg /mL of crude lipase, 0.8 mL of acyl donor (isopropenyl acetate or vinyl acetate) and 0.1% of water. The reaction was carried out in a 50 mL Teflon flask at 28°C and 250 RPM on a rotary shaker.

Semi-preparative scale acetylation of I

The reaction mixture in 2 L of MEK contained 4 g substrate **1**, 40 g crude lipase AK, 160 mL vinyl acetate, and 0.1% water. The reaction was carried out in a glass reactor at 28°C and 200 RPM. After a 10-day reaction period, the enzyme was recovered by filtration, washed with 200 mL of MEK and air dried for future use. Combined organic solvent (MEK) was removed under reduced pressure to obtain 12.4 g of a light yellowish liquid. The oily material was washed with hexane, dissolved in 700 mL of ethyl acetate, washed with water and brine and evaporated under reduced pressure to give about 8 g of material. Chromatography of this material on a silica gel column gave 1.5 g of S-(−)-acetate **2** and 0.98 g of (+)-alcohol **1**. The chemical purity of both chiral acetate **2** and alcohol **1** were >98% as analyzed by HPLC. The optical purity of S-(−)-acetate **2** was 98%. $[\alpha]_D^{25}=-6.2$ (C 1, CH_2Cl_2). ^1H NMR, 300 MHz, ($CDCl_3$) d 1.7–2.0 (m 8H); 2.2 (S, 3H acetyl); 3.5–3.9 (m, 2H, NCH_2); 5.05 and 5.09 (S-benzyl CH_3); 7.33 (10 H, aromatic).The optical purity of (+)-alcohol **1** was 98.8%. ^1H NMR, 300 MHz, ($CDCl_3$) d 1.7–2.0 (m 8H); 3.5–3.9 (m, 2H, NCH_2); 5.05 and 5.09 (S-benzyl CH_3); 7.33 (10 H, aromatic). The absolute configuration of chiral **2** is S-(−)-acetate **2**.

Analytical methods

Quantitation of substrate **1**, and acetate **2** was carried out using a Hewlett Packard HPLC. A Hypersil ODS column (100×4.5 mm, ID 5 m) was used at 37°C. The mobile phase was 40% isopropanol in water, the flowrate was 0.5 mL/min and the detection wavelength was 240 nm. The retention times for **1** and **2** were 18.7 and 25.01 min, respectively. The resolution of enantiomers of racemic acetate and racemic alcohol were carried out using a Chiralpak AD (25 cm×4.6 mm, 5 m ID, Diacel Chemical Ind. Ltd) column at 28°C. The mobile phase was 97.5% hexane, 1.5% ethyl alcohol and 1% cyclohexanol. The flow rate was 0.8 mL/min and the detection wavelength was 254 nm. Under above conditions, the retention times for enantiomers of racemic acetate were 22.18 min and 23.97 min. The retention times for enantiomers of alcohol were 26.7 min and 39.15 min.

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