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Stereoselective acetylation of [1-(hydroxy)-4-(3-phenyl)butyl]phosphonic acid, diethyl ester

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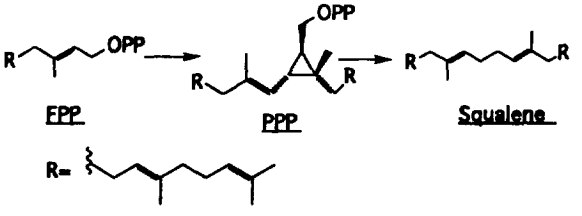
Abstract: The chiral intermediate (S) [1-(acetoxyl)-4-(3-phenyl)butyl]phosphonic acid, diethyl ester **2** was prepared for the total synthesis of a squalene synthase inhibitor, BMS-188494. The stereoselective acetylation of racemic [1-(hydroxy)-4-(3-phenyl)butyl] phosphonic acid, diethyl ester **1** was carried out in toluene using lipase from *Geotrichum candidum*. Isopropenyl acetate was used as an acylating agent. A reaction yield of 38% and an optical purity of 95% were obtained for chiral **2**. © 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] Squalene synthase is the first pathway-specific enzyme in the biosynthesis of cholesterol and catalyzes the head-to-head condensation of two molecules of farnesyl pyrophosphate (FPP) to form squalene. It has been implicated the transformation of FPP into presqualene pyrophosphate (PPP),[9] however the precise mechanism of the enzymatic conversion is not fully understood.



FPP analogues are a major class of inhibitors of squalene synthase.[10–12] However, this class of compounds lack specificity and are potential inhibitors of other FPP consuming transferases such as geranyl geranyl pyrophosphate synthase and protein farnesyl transferase. To increase enzyme specificity, analogues of PPP and other mechanism based enzymes inhibitors have been synthesized.[13–15] BMS-188494 is a potent squalene synthase inhibitor effective as an anticholesterol drug.[16,17]

In this report we describe the lipase-catalyzed stereoselective acetylation of **1** to chiral **2**. Chiral acetate **2** is a key intermediate for the total chemical synthesis of BMS-188494.

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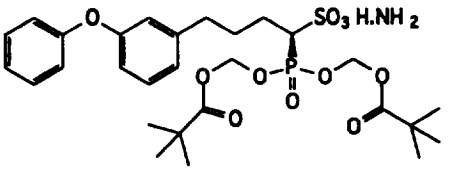
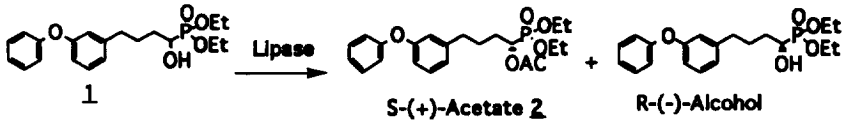
Table 1. Stereoselective acetylation of **1** by *Geotrichum candidum* lipase

Enzyme added (mg/mL)	Substrate added (mg/mL)	Reaction time (hr)	Substrate 1 (mg/mL)	Product 2 (mg/mL)	Yield of 2 (%)	Optical purity of 2 (%)
20	1	110	0.776	0.14	14	95
50	1	24	0.84	0.16	16	95
		100	0.62	0.34	34	95
50	2.5	42	1.45	0.55	27	94
		100	1.7	0.77	31	94
		124	1.63	0.81	32	94
50	5	42	4	1	20	93
		100	3.7	1.3	26	94
		124	3.61	1.4	27	94



BMS-188494



1 S-(+)-Acetate **2** + R-(-)-Alcohol

Results and discussion

Commercially available lipases from *Candida cylindraceae*, *Rhizopus niveus* (lipase N), *Penicillum* sp. (lipase F12), *Aspergillus usamii* (lipase F10), *Mucor* sp. (lipase MAP-10), Porcine pancreatic lipase, *Pseudomonas cepacia* (lipase PS-30) and *Geotrichum candidum* lipase were screened for the stereoselective acetylation of racemic **1** in an organic solvent (toluene) in the presence of isopropenyl acetate as acyl donor. *Geotrichum candidum* lipase efficiently catalyzed the enantioselective acetylation of racemic **1**. The reaction yields and optical purities of **2** obtained with various levels of enzyme and substrate **1** are as shown in the Table 1. Using 1 or 2.5 mg/mL substrate and 50 mg/mL of crude lipase, the reaction yield of about 32–34% and an optical purity of 95% were obtained. Lower reaction yield (27%) was obtained using 5 mg/mL substrate concentration.

Effect of solvents was evaluated in the acetylation of **1** by *Geotrichum candidum* lipase at 40°C for 96 h reaction period. When the reactions were catalyzed in hexane, tert-butyl methylether, methyl ethyl ketone, tetrahydrofuran, acetone, and dichloromethane, no product was detected and the reaction was inhibited. Only toluene and hexane:tert-butyl methylether mixture (1:1) were effective solvents in supporting the acetylation of **1** to acetate **2**. The reaction yields of 25% and 38% and optical purities of 82% and 95% were obtained using hexane:tert-butyl methylether mixture (1:1) and toluene as solvent, respectively.

The effect of temperature on the acetylation of **1** by *Geotrichum candidum* lipase was evaluated in toluene (Table 2). The optimum temperature was 37–40°C. The reaction yield of about 36–38% and optical purity of 95% were obtained for chiral acetate **2**.

Table 2. Effect of temperature on the acetylation of **1** by *Geotrichum candidum* lipase

Temperature	Yield of **2** (%)	Optical purity of **2** (%)
28	14	92.5
37	36	95
40	37	95
45	33	87
50	33	77

Reactions were carried out in 9.9 mL of toluene containing 2mg/mL of substrate, 50 mg/mL of enzyme, 0.11 mL of isopropenyl acetate and 0.005 mL of water at . designated temperature for 96 hrs.

The effect of water concentration on the acetylation of **1** by *Geotrichum candidum* lipase was evaluated in toluene. The optimum water concentraion was 0.05%. The reaction yield of 38% and an optical purity of 95% were obtained for chiral acetate **2**. At 0.1% and 0.025% water levels, the reaction yields were 16% and 8%, respectively. Reactions were carried out for a period of 120 h.

Vinyl acetate was used as an alternate acyl donor in the acetylating of **1** by *Geotrichum candidum* lipase in toluene. A reaction yield of 33% and an optical purity of 95% was obtained over a reaction period of 120 h.

Enzymatic acetylation of **1** by *Geotrichum candidum* lipase was scaled-up to a 2 L batch as described in the Experimental section. After 120 h, a yield of 40% and optical purity of 95% were obtained for desired chiral acetate **2**. From the reaction mixture, 0.85 g of S-(+)-acetate **2** was isolated in overall 38% yield (theoretical max. yield is 50%). An optical purity of 95% and a chemical purity of 98% were obtained for the isolated S-(+)-acetate. A specific rotation $[\alpha]_D$ of +7.5 (C=1 in CH_2Cl_2) was obtained for desired chiral acetate **2**.

Both stereoselective and regioselective acylation reactions have been conducted to prepare intermediates for chemical synthesis.[18] 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 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.[19] Hirtake *et al.* used vinyl acetate as an acyl donor for enzymatic transesterification reactions.[20] In such reactions, 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.*[19] Lipase-catalyzed transesterification of alcohols, glycerol derivatives, and sugars using enol esters as the acyl donor has been described by Wang *et al.*[21,22] 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.

Chiral α-hydroxyphosphonates are useful precursors for α-aminophosphonic acid which have received considerable medical attention in recent years due to their potential biological activities as analogues of amino acids.[23,24] Lipase-catalyzed enantioselective hydrolysis of α-(acyloxy)phosphonates has been demonstrated by Li and Hammerschmidt.[25] In this report we have described the lipase-catalyzed stereoselective acetylation of racemic [1-(hydroxy)-4-(3-phenyl)butyl]phosphonic acid, diethyl ester **1** to prepare (S)[1-(acetoxyl)-4-(3-phenyl)butyl]phosphonic acid, diethyl ester **2**. Chiral **2** was prepared for the total synthesis of a squalene synthase inhibitor, BMS-188494.

Experimental

Starting substrate **1** and reference compounds racemic alcohol and chiral **2**, were synthesized by colleagues in the Chemical Process Research Department, Bristol-Myers Squibb Pharmaceutical Research Institute as described previously.[16,17] 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 lipase from *Candida cylindraceae* was purchased from Baiocatalyst (UK), lipases from *Rhizopus niveus* (lipase N), *Mucor* sp. (lipase MAP-10), *Pseudomonas cepacia* (lipase PS-30) and *Geotrichum candidum* lipase were purchased from Amano International (USA). *Penicillum* sp. (lipase F12) and *Aspergillus usamii* (lipase F10) were purchased from Enzymatics (UK). Porcine pancreatic lipase was purchased from Sigma Chemicals (USA).

Acetylation of 1 by lipase

Typically, the reaction was carried out in 9.9 mL of toluene (dried over 4 Å molecular sieves) using 1 mg/mL of substrate **1**, 50 mg/mL of crude lipase, 0.11 mL of isopropenyl acetate, and 0.005 mL of water. The reaction was carried out in a 50 mL Teflon flask at 40°C and 250 RPM on a rotary shaker. All solvents used to conduct reactions were dried over 4 Å molecular sieves.

Semi-preparative scale acetylation of 1

The reaction mixture in 2 L of toluene contained 2.2 g substrate **1**, 63 g crude *Geotrichum candidum* lipase, 24.4 mL isopropenyl acetate, and 0.55 mL water. The reaction was carried out in a glass reactor at 37°C and 200 RPM. After 120 h reaction period, the enzyme was recovered by filtration, washed with 200 mL of toluene and air dried for future use. Combined organic solvent (toluene) was removed under reduced pressure to obtain a 2.4 g of a light brown liquid. Chromatography of this material on a 100 g silica gel (prewashed with 400 mL of ethyl acetate:hexane, 80:20 mixture) column gave 0.85 g of S-(+)-acetate **2** as colorless oil, when column was eluted with ethyl acetate:hexane:triethylamine (80:17:3) mixture. R-(−)-Alcohol was subsequently eluted with the same solvent. The chemical purity of both acetate and alcohol were >98% as analyzed by HPLC. The optical purity of chiral acetate **2** was 95%. $[\alpha]_D^{25}$=+7.5 (C 1, CH_2Cl_2). ^{1}H-NMR, 300 MHz, ($CDCl_3$) d 1.2 [m, 6H, $P(OCH_2CH_3)_2$]; 1.45 (m, 2H); 1.5 (m, 2H); 2.15 (S, 3H, acetyl); 2.6 (m, 2H); 4.15 [m, 4H, $P(OCH_2CH_3)_2$]; 5.31 (m, 1H) 6.75–7.2 (m, 9H). MS $(M+H)^+$ 421; Anal. Calcd. for $C_{22}H_{29}O_6P$ C, 62.85; H, 6.95; P, 7.37; Found: C, 62.81; H, 7.05; P, 7.69.

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 flowrate 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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