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Preparation of a chiral synthon for an HBV inhibitor: enzymatic asymmetric hydrolysis of (1α,2β,3α)-2-(benzyloxymethyl)cyclopent-4-ene-1,3-diol diacetate and enzymatic asymmetric acetylation of (1α,2β,3α)-2-(benzyloxymethyl)cyclopent-4-ene-1,3-diol

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Abstract—Enantioselective asymmetric hydrolysis of (1α,2β,3α)-2-(benzyloxymethyl)-cyclopent-4-ene-1,3-diol diacetate **1** to the corresponding (+)-monoacetate **2** was carried out using lipase PS-30 from *Pseudomonas cepacia* or pancreatin. A reaction yield of 85 M % with an enantiomeric excess (ee) of 98% was obtained. Using pancreatin, a reaction yield of 75 M % with an ee of 98.5% was obtained. Asymmetric acetylation of (1α,2β,3α)-2-(benzyloxymethyl)-cyclopent-4-ene-1,3-diol **3** to the corresponding (−)-monoacetate **4** was carried out using lipase PS-30 with isopropenyl acetate as the acylating agent. A reaction yield of 80 M % with an ee of 98% was obtained for (−)-monoacetate **4**.

1. Introduction

The current interest in the enzymatic production of chiral compounds lies in the preparation of intermediates for pharmaceutical synthesis.[1–11] Lipases have been used extensively in the preparation of non-racemic chiral compounds by enantioselective enzymatic hydrolysis, esterification, and transesterification reactions.[12–24]

Previously we have demonstrated the asymmetric enzymatic hydrolysis of 2-cyclohexyl- and 2-phenyl-1,3-propanediol diacetate.[23] The chiral monoacetates obtained are key intermediates for the synthesis of the angiotensin converting enzyme (ACE) inhibitor Monopril®. The asymmetric hydrolysis of (*exo*,*exo*)-7-oxabicyclo[2.2.1]-heptane-2,3-dimethanol diacetate ester to the corresponding monoacetate ester has been demonstrated with lipase PS-30.[24] The chiral monoacetate ester is a key intermediate in the synthesis of a thromboxane A2 antagonist. Enzymatic hydrolysis of the (1α,2β,3α)-2-(benzyloxymethyl)cyclopent-4-ene-1,3-diol diacetate has been demonstrated by Griffith and Danishefsky to produce the corresponding monoester using the acetylcholine esterase from the electric eel[25,26] and pocine pancreatic lipase by Legrand and Roberts.[27] An efficient synthesis of the carbocyclic nucleosides (−)-aristeromycin and (−)-neplanocin A has been developed in an enantioselective manner starting from the Diels–Alder adduct of cyclopentadiene and dimethyl acetylenedicarboxylate. The symmetric unsaturated dimethyl ester, dimethyl (3aα,4β,7β,7aα)-3a,4,7,7a-tetrahydro-2,2-dimethyl-4,7-methano-1,3-benzodioxole-5,6-dicarboxylate was quantitatively hydrolyzed with pig liver esterase to yield a half-ester with reasonably high ee (88%).[28] Deardorff et al. have demonstrated the hydrolysis of *cis*-3,5-diacetoxycyclopent-1-ene to the corresponding (3*R*,5*S*)-acetoxy-5-hydroxycyclopent-1-ene using electric eel acetylcholinesterase in 94% yield with an ee of 96%.[29] Pig liver esterase and porcine pancreatic lipase (PPL) catalyzed the enantiotopic selective hydrolysis of 2,5-bis(methoxy-carbonyl) and 2,5-bis(acetoxymethyl)

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meso-diester derivatives of 3,4-(isopropylidenedioxy)-tetrahydrofuran to the corresponding monoesters with an ee of 72% being demonstrated by Hultin et al.[30] Transesterification of the 2,5-bis(hydroxymethyl) derivatives with trifluoroethyl laurate by PPL in ether also showed stereoselectively but in the opposite stereochemical sense from the hydrolysis of the corresponding diacetate.[31] The synthesis of 3′-deoxycarbacyclic nucleoside analogues has recently been demonstrated by the regioselective ring opening of the cyclopentene epoxide with lithium di-*n*-propylamide in ether.[32]

Herein we report the enantioselective asymmetric hydrolysis of (1α,2β,3α)-2-(benzyloxymethyl)cyclopent-4-ene-1,3-diol diacetate **1** to the corresponding (+)-monoacetate **2** by lipase PS-30 and pancreatin. We also describe the enzymatic asymmetric acetylation of (1α,2β,3α)-2-(benzyloxymethyl)cyclopent-4-ene-1,3-diol **3** to the corresponding (−)-monoacetate **4** using lipase PS-30 (Fig. 1). Chiral monoacetate esters **2** and **4** are key intermediates in the total synthesis of entecavir, recently approved for treatment of hepatitis B viral infection.[33–37] Entecavir is a carboxylic analogue of 2′-deoxyguanosine in which the furanose oxygen is replaced with an exocyclic double bond (Fig. 1).

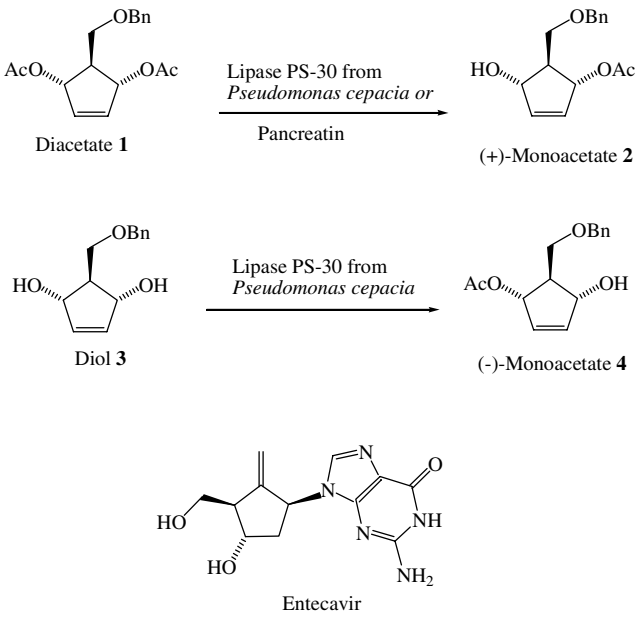

Figure 1.

2. Results and discussion

Various lipases and the acetylcholine esterase from the electric eel were screened for the enzymatic hydrolysis of (1α,2β,3α)-2-(benzyloxymethyl)cyclopente-4-ene-1,3-diol diacetate **1** to the corresponding (+)-monoacetate **2**. Among the enzymes evaluated, pancreatin, the immobilized lipase PS-30 from *Pseudomonas cepacia* and acetylcholine esterase all catalyzed the asymmetric hydrolysis of diacetate **1** to the corresponding (+)-monoacetate **2**. Lipase MAP-10, penicillin V amidase, and lipase from *Rhizopus* sp. did not hydrolyze diacetate **1**. Further experimentation was carried out using lipase PS-30 and acetylcholine esterase. Preparative scale hydrolysis of diacetate **1** was carried out in a 2-L reactor with a substrate input of 26 g/L (total input 52 g) and 90 g/L input of immobilized lipase PS-30. After 18 h reaction time, 31.2 g of (+)-monoacetate **2** was obtained with 78 M % yield with an ee of 98% (Table 1). A preparative batch for the hydrolysis of diacetate **1** to the corresponding monoacetate was carried out using acetylcholine esterase. The reaction was carried out using a 5 g/L substrate input and 2400 units of enzyme input in a 0.6 L volume. After 50 h reaction time, 2 g (82 M % yield with 98% ee) of (+)-monoacetate **2** was obtained (Table 1).

We also screened various lipases for the enantioselective acylation of *meso*-diol **3** to the corresponding monoacetate using isopropenyl acetate as an acyl donor. Pancreatin and lipase PS-30 from *P. cepacia* catalyzed the enantioselective acylation of *meso*-diol **3** to the corresponding (−)-monoacetate **4**. Kinetics of the acylation reaction using lipase PS-30 are shown in Table 2.

Griffith and Danishefsky reported the asymmetric hydrolysis of (1α,2β,3α)-2-(benzyloxymethyl)cyclopent-4-ene-1,3-diol diacetate **1** by electric eel acetylcholinesterase during the total synthesis of allosamidin, a chitin synthase inhibitor potentially useful for preventing fungal and insect growth.[25] Acetylcholinesterase is an expensive enzyme, which is not readily available in large quantities. Lipase PS-30 is inexpensive and readily available in large quantities. Immobilized lipase PS-30 used in the asymmetric hydrolysis of diacetate **1** can be recovered and reused for many cycles. We have prepared both (+)-monoacetate **2** and (−)-monoacetate **4** by lipase PS-30 catalyzed hydrolysis of diacetate **1** or acylation of diol **3**, respectively, in very high yield and ee.

Table 1. Enzymatic asymmetric hydrolysis of diacetate **1**

Enzyme	Reaction time (h)	Diacetate **1** (g/L)	Monoacetate **2** (g/L)	Diol (g/L)	Monoacetate **2** M % yield	% ee of (+)-monoacetate
Immobilized	0.1	26	1.2	0.03		
Lipase PS-30	18	0.1	17.6	3.4	80	98
Acetylcholine	0.1	2.85	0.56	Trace		
Esterase	50	0	2.0	0.09	82	98

Reactions were carried out as described in the Experimental section.

Table 2. Enzymatic asymmetric acetylation of diol **3** by immobilized lipase PS-30

Reaction time (h)	Monoacetate **4** (g/L)	Monoacetate **4** M % yield	% ee of (−)-monoacetate **4**
24	8.2	36	98.9
48	15.4	62	99
72	22.5	90	99

3. Experimental

The physico-chemical properties including spectral characteristics (^{1}H NMR, ^{13}C NMR, mass spectra) were in full accordance 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. Racemic monoacetate **2** required as an HPLC reference standard was prepared by base-catalyzed deacetylation of **1** with potassium carbonate in methanol.

3.1. (1α,2β,3α)-2-(Benzyloxymethyl)cyclopent-4-ene-1,3-diol diacetate 1

A mixture of (1α,2β,3α)-2-(benzyloxymethyl)cyclopent-4-ene-1,3-diol[35] **3** (2.2 g, 9.9 mmol), acetic anhydride (4 mL), and pyridine (2.5 mL) was stirred at room temperature for 12 h. The reaction mixture was concentrated under vacuum and the residue chromatographed (silica gel, 30% ethyl acetate in hexanes) to provide diacetate **1** (2.8 g, 92%) as a light yellow oil.

3.2. Source of enzymes

Lipase PS-30, lipase MAP10, and lipase from *Rhizopus* sp. were obtained from Amano International Enzyme Company. Pancreatin and acetylcholin esterase was obtained from Sigma Chemicals. Lipase PS-30 was immobilized on the hydrophobic resin Accurel polypropylene as described earlier.[12]

3.3. Enzymatic hydrolysis of diacetate 1

Enantioselective asymmetric hydrolysis of (1α,2β,3α)-2-(benzyloxymethyl)cyclopent-4-ene-1,3-diol diacetate **1** was carried out as described below.

The reaction mixture contained 9 mL of 25 mM pH 7.0 potassium phosphate buffer pH 7.0, 1 mL toluene, 2.5 mg/mL diacetate **1**, and 50 mg/mL of enzyme. The reaction was carried out at 25 °C and the pH of the reaction mixture maintained at 7.0 by the addition of 1 M NaOH as required. The progress of the reaction was monitored by high pressure liquid chromatography. Enantioselective asymmetric hydrolysis of diacetate **1** was carried out using pancreatin or lipase PS-30 from *P. cepacia*. The reaction mixture in 40 mL contained 36 mL of 25 mM pH 7.0 potassium phosphate buffer, 4 mL toluene, 2.5 mg/mL diacetate **1**, and 50 mg/mL of lipase PS-30 or pancreatin. The reaction was carried out at 25 °C and the pH of the reaction mixture maintained at 7.0 by addition of 1 M NaOH as required. The progress of the reaction was monitored by high pressure liquid chromatography. The reaction was terminated after about 16 h when the remaining substrate **1** concentration had dropped below 0.05 mg/mL. The reaction mixture was extracted with two volumes of ethyl acetate. The organic phase was separated and concentrated under reduced pressure to obtain (+)-monoacetate **2**, which was purified by flash chromatography. When the hydrolysis was performed using lipase PS-30, (+)-monoacetate **2** was obtained in 85 M % yield with 98% ee. When the hydrolysis was performed using pancreatin, (+)-monoacetate **2** was obtained in 75 M % yield with 98.5% ee.

The semi-preparative scale asymmetric hydrolysis of diacetate **1** was carried out using lipase PS-30 from *P. cepacia* immobilized on Accurel polypropylene. The reaction mixture in 2 L contained 1.8 L of 25 mM pH 7.0 potassium phosphate buffer, 200 mL toluene, 52 g diacetate **1**, and 180 g immobilized lipase PS-30. At the end of the reaction (18 h), the immobilized enzyme was filtered and the clear supernatant obtained was extracted with two volumes of ethyl acetate. The separated organic phase was concentrated under reduced pressure to provide the desired product, which was purified by flash chromatography, (+)-monoacetate **2** (32.5 g), in 80 M % yield with 98% ee.

3.4. Enzymatic acetylation of diol 3

Enantioselective acetylation of diol **3** was carried out using various enzymes. The reaction mixture in 10 mL of heptane/*tert*-butyl methyl ether (10:1) contained 200 mg of diol **3**, 50 mg of enzyme, and 1.1 M equiv of isopropenyl acetate. The reaction was carried out at 33 °C in a 50-mL Teflon flask. The progress of the reaction was monitored by high pressure liquid chromatography.

Semi-preparative scale acetylation of diol **3** was carried out using immobilized lipase PS-30 from *P. cepacia.* The reaction mixture in 1 L of heptane/MTBE (10:1) contained 25 g of diol **3**, 25 g of immobilized lipase PS-30, and 2 M equiv of isopropenyl acetate as acylating agent. The reaction was carried out at 33 °C. The progress of the reaction was monitored by HPLC. The reaction was terminated when the remaining substrate **3** concentration was below 0.1 mg/mL. After 72 h of reaction time, the product (−)-monoacetate **4** was isolated in 80 M % yield with 98% ee.

4. Analytical methods

Samples from enzymatic reactions were extracted with three volumes of ethyl acetate. The solvent layer was collected and evaporated under vacuum, and the residue dissolved in mobile phase before analysis. Analysis of diacetate **1**, diol **3**, and the monoacetate were carried

out using HPLC with a Kromasil C-8 column (100 × 4.5 mm, ID 5 m). Mobile phases A and B were used in a gradient; solvent A: 25 mM KH_2PO_4 (pH 7.0), solvent B: acetonitrile.

Time (min)	Solvent A (%)	Solvent B (%)
0	70	30
30	25	75
35	25	75
40	70	30

The flow rate was 1 mL/min and the detection wavelengths were 210, 250, and 280 nm. The retention times for diacetate **1**, diol **3**, and the monoacetate were 30.3, 12, and 20 min, respectively. The ee of the monoacetate was determined by chiral HPLC. A Bakerbond Chiralpak AD column (100 × 4.5 mm, ID 5 m) was used at 40 °C; injection volume was 10 μL; mobile phase was a 95% heptane/5% isopropanol mixture; flow rate was 1 mL/min; and detection wavelength was 210 nm. The retention times for compounds (+)-monoacetate **2** and (−)-monoacetate **4** were 15 and 22 min, respectively.

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