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Preparation of chiral synthon for HIV protease inhibitor: stereoselective microbial reduction of N-protected α-aminochloroketone

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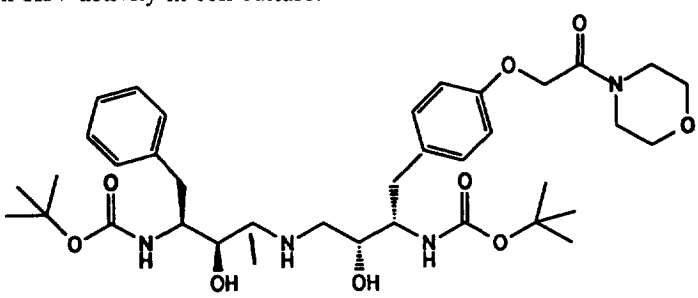
Abstract: The chiral intermediate (1S,2R) [3-chloro-2-hydroxy-1-(phenylmethyl)propyl] carbamic acid, 1,1-dimethylethyl ester **2a** was prepared for the total synthesis of an HIV protease inhibitor, BMS-186318. The stereoselective reduction of (1S) [3-chloro-2-oxo-1-(phenylmethyl)propyl] carbamic acid, 1,1-dimethyl-ethyl ester **1** was carried out using microbial cultures among which *Streptomyces nodosus* SC 13149 efficiently reduced **1** to **2a**. A reaction yield of 80% was obtained. The optical purity of 99.8% and the diastereomeric purity of 99% were obtained for chiral alcohol **2a**. © 1997 Elsevier Science Ltd

Introduction

The current interest in enzymatic production of chiral alcohols lies in the preparation of intermediates for pharmaceutical synthesis.[1–8] Oxidoreductases from yeast,[4–9] horse liver,[10] *Thermoanaerobic brockii*,[11,12] *Lactobacillus kefir*,[13] *Pseudomonas* sp.,[14] *Geotrichum candidum*,[15,16] *Hansenula polymorpha*,[17,18] *Mortierella rammaniana*,[19] and *Sulfolobus solfataricus*[20] have been used in the synthesis of chiral alcohols.

An essential step in the life cycle of human immunodeficiency virus (HIV-1) is the proteolytic processing of its precursor proteins. This processing is accomplished by HIV-1 protease, a virally encoded enzyme. Inhibition of HIV-1 protease arrests the replication of HIV in vitro. Thus, HIV-1 protease is an attractive target for chemotherapeutic intervention. Recently Barrish *et al.*[21] reported the discovery of a new class of selective HIV protease inhibitors which incorporates a C_2 symmetric aminodiol core as its key structural features. Members of this class and particularly BMS-186318 display potent anti-HIV activity in cell culture.



BMS-186318 (Antiviral agent)

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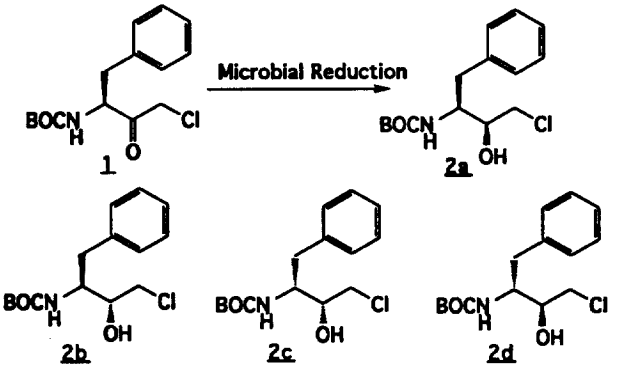
Table 1. Stereoselective microbial reduction of ketone **1**

Microorganisms	Reaction yield of 2a (%)	Diastereomeric purity of 2a (%)	Optical purity of 2a (%)
Streptomyces nodosus SC 13149	56	>99	99.9
Pullularia pullulans SC 13849	47	81	99.9
Candida boidinii SC 13821	39	>99	99.9
Nocardioides albus SC 13910	5	85	99.9
Mortierella ramanniana SC 13850	54	91	99.9
Caldariomyces fumigo SC 13901	51	93	99.9

In this report we describe the stereoselective microbial reduction of (1S) [3-Chloro-2-oxo-1-(phenylmethyl)propyl] carbamic acid, 1,1-dimethyl-ethyl ester **1** to **2a**. Chiral alcohol **2a** is a key intermediate for the total chemical synthesis of BMS-186318.



Results and discussion

About 100 microorganisms were screened for the stereoselective reduction of **1** to **2a**. The reaction yields, diastereomeric purity and optical purity of **2a** obtained with the best six cultures are as shown in the Table 1. Lower diastereomeric purities (<70%) and reaction yields (<20%) were obtained with other cultures. The reaction yield and diastereomeric selectivity were dependent upon the microorganism used during the reduction of **1**. *Streptomyces nodosus* SC 13149, *Candida boidinni* SC 13821, *Mortierella ramanniana* SC 13850, and *Caldariomyces fumago* SC 13901 gave >39% reaction yields, >91% diastereomeric purities and 99.9% optical purity of product **2a**.

Further research was conducted using *Streptomyces nodosus* SC 13149 and *Mortierella ramanniana* SC 13850 to convert ketone **1** to the corresponding chiral alcohol **2a**. Cells of *Streptomyces nodosus* SC 13149 and *Mortierella ramanniana* SC 13850 were grown in a 25-L fermentor for 48 hours. Cells were collected and suspended in 100 mM potassium phosphate buffer pH 6.8 and the resulting cell-suspensions were used to carry out the two-stage process for biotransformation of **1** as described in the experimental section. After 24 h biotransformation, a reaction yield of 67%, optical purity of 99.9% and diastereomeric purity of >99% were obtained for chiral alcohol **2a** using cells of *Streptomyces nodosus* SC 13149. *Mortierella ramanniana* SC 13850 gave a reaction yield of 54%, optical purity of 99.9% and diastereomeric purity of 90% for chiral alcohol **2a** (Table 2).

A single-stage fermentation–biotransformation process was developed for conversion of ketone **1**

Table 2. Stereoselective microbial reduction of ketone **1**: Two-stage process

Microorganism	Reaction Time (Hours)	Compound 2a (g/L)	Yield (%)	Diastereomeric purity of 2a (%)	Optical purity of 2a (%)
Streptomyces nodosus SC 13149	12	0.56	56		
	24	0.67	67	>99	99.9
Mortierella ramanniana SC 13850	12	0.22	22		
	24	0.54	54	91	99.9

Table 3. Stereoselective microbial reduction of ketone **1**: Single-stage process

Reaction Time (Hours)	Compound 2a (g/L)	Yield (%)	Diastereomeric purity of 2a (%)	Optical purity of 2a (%)
Streptomyces nodosus SC 13149				
24	0.56	56		
48	0.80	80	>99	99.9

to chiral alcohol **2a** with cells of *Streptomyces nodosus* SC 13149 as described in the Experimental section.

A reaction yield of 80%, diastereomeric purity of >99% and an optical purity of 99.8% (Table 3). From 12-L reaction mixture, 6.5 g of chiral alcohol **2a** was isolated as white needle crystals in overall 62% yield. The diastereomeric purity and the optical purity of the isolated chiral alcohol were >99% and >99.8%, respectively.

The synthetically useful alcohol dehydrogenases from yeast,[4–6,8] horse liver,[10] and secondary alcohol dehydrogenase[11,12] usually reduce carbonyl compounds to give an (S)-alcohol. Recently, Bradshaw *et al.* have demonstrated the use of alcohol dehydrogenase from *Lactobacillus kefir*[13] and *Pseudomonas* sp.[14] as catalyst for synthesis of chiral aromatic, cyclic, and aliphatic alcohols from their corresponding ketones.

In our continuing effort to prepare chiral synthon for drugs development, we have demonstrated the microbial reduction N-(4-(1-oxo-2-chloroacetyl ethyl) phenyl methane sulfonamide to the corresponding chiral alcohol. R-(+)-Alcohol is an intermediate for the synthesis D-(+)-sotalol, a β-3-receptor antagonist.[17] Preparation of R-(+)-BMY-14802, an effective antipsychotic drug by the stereoselective reduction of 1-(4-fluorophenyl)-4-[4-(5-fluoro-2-pyrimidinyl)-1-piperazinyl]butan-1-one by *Mortierella ramanniana* ATCC 38191 has been demonstrated.[19] We have also demonstrated the stereoselective reduction of 4,5-dihydro-4-(4-methoxyphenyl)-6-(trifluoromethyl-1H-1)-benzazepin-2-one to the corresponding chiral alcohol, an intermediate for the effective calcium antogonist.[22] Recently, we have purified an oxidoreductase (MW 900,000) from *Geotrichum candidum* which catalyzes the reduction of 4-chloro-3-oxobutanoic acid ethyl ester to the corresponding S-(−) hydroxy ester, a chiral intermediate needed for the total synthesis of a anticholesterol agent.[23] In this report, we have described the preparation of chiral alcohol **2a** by stereoselective microbial reduction. Chiral alcohol is an intermediate for antiviral agent.

Experimental

Starting substrate **1** and reference compounds **2a–d**, were synthesized by colleagues in the Chemical Process Research Department, Bristol-Myers Squibb Pharmaceutical Research Institute as described previously.[21] 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.

Microorganisms

Microorganisms (Table 1) were obtained from the culture collection of the Bristol-Myers Squibb Pharmaceutical Research Institute and from the American Type Culture Collection, Rockville, MD. Microbial cultures were stored at −90°C in vials.

Growth of microorganisms

For screening purposes, one vial of each culture was used to inoculate 100 mL of medium A containing 1% malt extract, 1% yeast extract, 2% glucose and 0.3% peptone. The medium was adjusted to pH 6.8 before sterilization. Cultures were grown at 28°C and 280 RPM for 48 hours. Cultures were harvested by centrifugation at 18,000 × g for 15 minutes, washed with 0.1 M potassium phosphate buffer pH 7.0, and used for reduction studies.

Reduction of 1 by cell-suspensions

Cells of various microorganisms were suspended separately in 100 mM potassium phosphate buffer (pH 7.0) at 20% (w/v, wet cells) cell concentration and supplemented with 1 mg/mL of **1** and 30 mg/mL of glucose. Reduction was conducted at 28°C and 150 RPM. Periodically, samples of 1 mL were taken and extracted with 5 mL of tert.butylmethylether:toluene (60:40). After centrifugation, the separated organic phase was collected and dried with a nitrogen stream. The oily residue obtained was dissolved in 1 mL of ethanol, filtered through a 0.2 μm LID/X filter and analyzed by HPLC.

Two-stage process for reduction of 1

Streptomyces nodosus SC 13149 and *Mortierella ramannina* SC 13850 culture were grown in a 25-liter fermentor containing 15 liter of medium A containing 0.025% UCON antifoam. Growth consisted of several inoculum development stages and fermentation. Inoculum development consisted of F1 and F2 stages. In the F1 stage, a frozen vial of each culture was inoculated into 100 mL of medium A contained in a 500-mL flask. Growth was carried out at 28°C and 280 RPM for 48 hours on a rotary shaker. In the F2 stage, 10 mL of F1 stage culture was inoculated into 1 L of medium A and incubated at 28°C and 280 RPM for 24 hours. Fermentors containing 15-liters of medium A were inoculated with 1 L of inoculum of each culture from a F2 stage. Fermentation was conducted at 25°C and 500 RPM with 15 LPM (liter per min) aeration for 48 hours. After 48 hours fermentation, cells were collected and stored at −90°C until further use. About 1 kg of wet cell pastes was collected from each fermentation.

Frozen cells from the above batches were used to conduct the reduction of **1** in a 5-liter reactor. Cell suspensions (10% w/v, wet cells) in 3 liter of 0.1 M potassium phosphate buffer (pH 6.0) were used. Compound **1** (3 gram) and glucose (30 gram) were added to the reactor and the reduction was carried out at 28°C and 160 RPM with 1 LPM aeration for 24 hours. The pH was maintained between 6.6 and 6.8. Periodically, samples were prepared as described above and analyzed by HPLC to determine the % conversion of **1** to **2a**. The diastereomeric purity and the optical purity of **2a** was determined by HPLC.

Single-stage process for reduction of 1

Streptomyces nodosus SC 13149 culture was grown in a 25-L fermentor as described above. After 30 hours of growth period, 15 g of ketone **1** was added to the fermentor and the biotransformation process was continued for 48 hours. The pH was maintained at 6.8 during biotransformation process.

Periodically, samples were prepared as described above and analyzed by HPLC to determine the % conversion of **1** to **2a**. The diastereomeric purity and the optical purity of **2a** was determined by HPLC.

Isolation of 2a

At the end of single-stage bioreduction, 12 L of the reaction mixture extracted with 24 L of tert.butylmethylether:toluene (60:40). The separated organic phase (20 L) was washed with 10 L of 0.1 M sodium chloride, dried over anhydrous sodium sulfate and evaporated under reduced pressure to obtain 7.5 gram of crude product which was recrystallized from ethyl acetate to obtain 6.5 g (62% overall yield) of white needle crystals of **2a**.The diastereomeric purity and the optical purity of the isolated chiral alcohol **2a** were >99% and >99.8%, respectively. ^{1}H-NMR ($CDCl_3$) d 1.4 (S,9H), 2.9 (d,2H, J=13Hz), 3.2 (S,1H); 3.6 (m,2H), 3.85 (m,1H), 4.5 (S,1H), 7.2–7.4 (m,5H). MS m/z 302 $(M+H)^+$ (calcd for $C_{15}H_{22}ClNO_2$, 301).

Analytical methods

Analysis of **1** and **2a–2d** were carried out using a Hewlett Packard HPLC. A YMC-PACK-ODS-A column (100×4.5 mm, ID 5 m) was used under the following conditions. Mobile phase was 10% methanol (solvent A) and 90% methanol (solvent B) used in a gradient:

Time (min)	Solvent A (%)	Solvent B (%)
0	100	0
25	25	75
25.2-30	100	0

The flow rate was 1 mL/min and the detection wavelength were 224, 250 and 280 nms.The retention times for substrate **1**, compounds **2a**, and **2c–2d** are 24 min, 23.5 min and 24.5 min, respectively. The optical purity of chiral alcohol **2a** was determined by chiral HPLC. A Bakerbond chiralpak AD column (100×4.5 mm, ID 5 m) was used at ambient temperature; injection volume was 10 μL; mobile phase was 97.5% hexane:1% cyclohexanol:1.5% ethyl acetate mixture; flow rate was 0.8 mL/min; and detection wavelength was 210 nm. The retention times for the compounds **2a**, **2b** and **2c–2d** were 14 min, 15.5 min and 21.5 min, respectively.

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