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Enzymatic preparation of (3*R*)-*cis*-3-acetyloxy-4-(1,1-dimethylethyl)-2-azetidinone: a side-chain synthon for an orally active taxane

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Abstract—The chiral intermediate (3*R*)-*cis*-3-acetyloxy-4-(1,1-dimethylethyl)-2-azetidinone **2** was prepared for the semi-synthesis of the new taxane **5**, an orally active anticancer compound. The enantioselective enzymatic hydrolysis of *cis*-3-acetyloxy-4-(1,1-dimethylethyl)-2-azetidinone **1** to the corresponding undesired (*S*)-alcohol **3** and unreacted desired **2** was carried out using immobilized lipase PS-30 or BMS lipase. Reaction yields of >48% and enantiomeric excesses of >99% were obtained for the desired **2**. Acetoxy β-lactam **2** was converted to hydroxy β-lactam **4** for use in the semisynthesis of **5**.

1. Introduction

Among the antimitotic agents, paclitaxel (Taxol®), a complex polycyclic diterpene, exhibits a unique mode of action on microtubule proteins responsible for the formation of the spindle during cell division.[1–4] Paclitaxel inhibits the depolymerization process of microtubulin.[5,6] Various types of cancers have been treated with paclitaxel and results in the treatment of ovarian cancer and metastatic breast cancer are very promising. Paclitaxel was originally isolated from the bark of the yew *Taxus brevifolia* and has also been found in other *Taxus* species in relatively low yield. Paclitaxel was obtained from *T. brevifolia* bark in 0.07% yield, requiring cumbersome purification from other related taxanes. It is estimated that about 20,000 pounds of yew bark (equivalent of about 3000 trees) are needed to produce 1 kg of purified paclitaxel.[1–4] The development of a semi-synthetic process for the production of paclitaxel by coupling of baccatin III (paclitaxel without the C-13 side-chain) and the C-13 side-chain has been demonstrated.[7] Due to the poor solubility of paclitaxel, various groups are involved in the development of water soluble taxane analogs.[8–16] Taxane **5** is a water soluble taxane derivative, which when given orally was as effective as i.v. paclitaxel in five tumor models [murine M109 lung and C3H mammary 16/C cancer, human A2780 ovarian cancer cells (grown in mice and rats) and HCT/pk colon cancer.[8] Compound **5** was also active in a human, hormone-dependent, prostate tumor model CWR-22 and just as effective as antiandrogen chemotherapy.[8]

Various approaches towards the synthesis of the C-13 paclitaxel side-chain from chiral synthons have been demonstrated, including the lipase catalyzed enantioselective esterification of methyl *trans*-β-phenylglycidate,[17] the enantioselective hydrolysis and transesterification of racemic esters and alcohols to prepare enantiomerically pure β-lactams[18] and the enantioselective microbial reduction of 2-keto-3-*N*-benzoylamino-3-phenyl propionic acid ethyl ester to yield (2*R*,3*S*)-(−)-*N*-benzoyl-3-phenyl isoserine ethyl ester.[19]

In this report we describe the enantioselective enzymatic hydrolysis of *cis*-3-acetyloxy-4-(1,1-dimethylethyl)-2-azetidinone **1** to prepare the C-13 side-chain synthon **2** of oral taxane **5** (Scheme 1).

2. Results and discussion

Commercially available lipases from *Pseudomonas cepacia* (lipase PS-30), *Geotrichum candidum* (lipase

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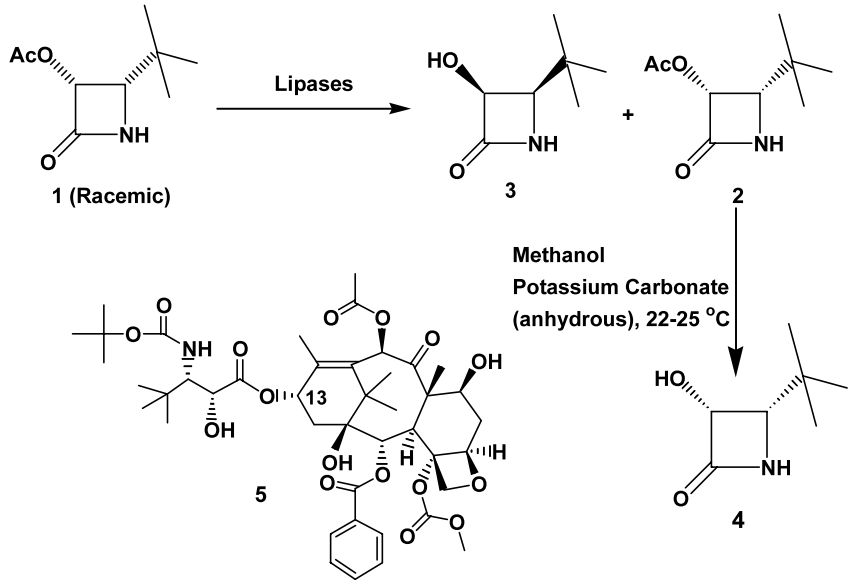

Scheme 1.

GC), *Rhizophus niveus* (lipase N), *Aspergillus niger* (lipase APF), and *Candida rugosa* (lipase AY) catalyzed the hydrolysis of the undesired enantiomer of racemic acetate **1** to the corresponding (*S*)-alcohol **3**, leaving the unreacted desired (3*R*)-*cis* acetate **2**. Reaction yields of 20–49% and enantiomeric excesses of 90–99% were obtained depending upon the enzyme (Table 1).

Table 1. Enantioselective hydrolysis of (3*R*)-*cis*-3-acetyloxy-4-(1,1-dimethylethyl)-2-azetidinone **1**

Lipases	Yield of desired unreacted **2** (%)	Enantiomeric excess of **2** (%)
Pseudomonas cepacia lipase PS-30	49	99
Geotrichum candidum lipase GC	40	97
Rhizopus niveus lipase N	32	92
Aspergillus niger lipase APF	20	90
Candida rugosa lipase AY	45	97
Pseudomonas sp. SC 13856 (BMS lipase)	48	99

Pseudomonas cepacia lipase PS-30 or BMS lipase were immobilized on accurel polypropylene and used in the resolution process. Various substrates were evaluated, among which (3*R*)-*cis*-3-acetyloxy-4-(1,1-dimethylethyl)-2-azetidinone was found to be the best. Table 2 demonstrates the results using Immobilized lipase PS-30. Similar results were obtained using immobilized BMS lipase.

Table 2. Evaluation of various substrates in enzymatic resolution process

Substrates	Unreacted ester (%)	Enantiomeric excess desired ester (%)
(3*R*)-*cis*-3-Acetyloxy-4-(1,1-dimethylethyl)-2-azetidinone	49.5	99.5
(3*R*)-*cis*-3-Propionyloxy-4-(1,1-dimethylethyl)-2-azetidinone	48	99
(3*R*)-*cis*-3-Hexanoyloxy-4-(1,1-dimethylethyl)-2-azetidinone	48	99
(3*R*)-*cis*-3-Benzyloxy-4-(1,1-dimethylethyl)-2-azetidinone	34	66
(3*R*)-*cis*-3-Phenylacetyloxy-4-(1,1-dimethylethyl)-2-azetidinone	32	54

Various amounts of immobilized lipase PS-30 were evaluated for the hydrolysis of substrate **1** in a 1-L bioreactor using same amount of substrate. Results are shown in Table 3. As expected, reaction time increased with decreasing enzyme input.

Preparative scale hydrolysis of (3*R*)-*cis*-3-acetyloxy-4-(1,1-dimethylethyl)-2-azetidinone was carried out using immobilized lipase 30 and BMS lipase as described in the Materials and Methods section. Results are shown in Table 4. In both batches, reaction yields of >48% and e.e.s of >99% were obtained for the desired acetate **2**. The desired acetoxy β-lactam **2** was hydrolyzed using

Table 3. Enantioselective hydrolysis of (3*R*)-*cis*-3-acetyloxy-4-(1,1-dimethylethyl)-2-azetidinone **1**: effect of enzyme input

Substrate input (g)	Enzyme input (g)	Reaction time (h)	Yield of **2** (%)	Enantiomeric excess of **2** (%)
30	30	3	49	99.2
30	15	4	49.4	99.5
30	7.5	6.5	49.4	99.5

Table 4. Preparative scale hydrolysis of (3*R*)-*cis*-3-acetyloxy-4-(1,1-dimethylethyl)-2-azetidinone **1**

Lipases	Substrate input (g)	Enzyme input (g)	Reaction time (h)	Yield of **2** (%)	Enantiomeric excess of **2** (%)
BMS-lipase	228	57	6.5	48.8	99.4
Lipase PS-30	229	57	6.5	49.4	99.5

potassium carbonate in methanol to the corresponding desired hydroxy β-lactam **4** and used in the synthesis of oral taxane **5**.

Chirality is a key factor in the efficacy of many drug products and agrochemicals, and thus the production of single enantiomers of chiral intermediates has become increasingly important in the pharmaceutical industry.[20] Single enantiomers can be produced by chemical or chemo-enzymatic synthesis. The advantages of biocatalysis over chemical synthesis are that enzyme-catalyzed reactions are often highly enantioselective and regioselective. A number of review articles[21–27] have been published on the use of enzymes in organic synthesis.

3. Conclusion

In this report we have described the enantioselective enzymatic hydrolysis of racemic acetate **1** to prepare chiral acetate **2**, which provides (3*R*)-*cis*-3-hydroxy-4-(1,1-dimethylethyl)-2-azetidinone **4**, the C-13 side-chain synthon required for the semi-synthesis of oral taxane **5**.

4. Experimental

4.1. Materials

Starting substrate **1** and reference compounds **2** and **3** were synthesized by colleagues in the Process Research and Development Department, Bristol-Myers Squibb Pharmaceutical Research Institute, as described previously.[28] The proton magnetic resonance (^{1}H NMR) and carbon magnetic resonance (^{13}C NMR) were recorded on a Brucker AM-300 spectrometer. The physico-chemical properties including spectral characteristics (^{1}H NMR, ^{13}C NMR, mass spectra) were in full accord for all these compounds as published previously.[28–30]

4.2. Lipase source

Commercially available lipases from *P. cepacia* (lipase PS-30), *Geotrichum candidum* (lipase GC), *Rhizophus niveus* (lipase N), *Aspergillus niger* (lipase APF), and *Candida cylindraceae* (lipase AY) were purchased from the Amano International Enzyme Company (Troy, Virginia, USA). BMS lipase was made by fermentation of *Pseudomonas* sp. SC 13856 as described previously.[31] Lipase PS-30 and BMS lipase were both immobilized on Accurel polypropylene (non-ionic polymeric adsorbent, 200–400 mesh polypropylene) as described previously.[31]

4.3. Enzymatic reaction

The enzymatic reaction mixture in 40 mL of 10 mM potassium phosphate buffer, pH 7.0, contained 10 mg/mL substrate and 5 mg/mL enzyme. The reaction was carried out at 40°C in a pH stat at pH 7.0 with agitation at 200 RPM. The reaction yield and enantiomeric excess of product was determined by high pressure liquid chromatography (HPLC).

4.4. Preparative scale hydrolysis and recovery of product

Preparative scale hydrolysis of racemic acetate **1** was carried out using immobilized lipase PS-30 or BMS lipase. In each case, the reaction mixture in 7.5 L of 10 mM potassium phosphate buffer (pH 7.0) contained 229 g of substrate and 57 g of the immobilized enzyme. The reaction was carried out at 40°C and 200 RPM in a 10-L fermentor for 390 min. The pH was maintained at 7.0 with 25% NaOH. At the end of the reaction, the immobilized enzyme, owing to its density, remained floating at the top of the liquid phase and was separated physically by draining the reaction mixture from the fermentor. The 7.5 L of the reaction mixture carried out using lipase PS-30 was split into two batches. Each batch (3.75 L) was extracted with ethyl acetate (2×2.6 L). The ethyl acetate layer was washed with brine (2×750 mL). The organic layer was dried over sodium sulfate. Removal of the ethyl acetate under reduced pressure on the rotary evaporator followed by drying under reduced pressure at ambient temperature (23–25°C) gave 178 g of the extracted β-lactams (1:1 mixture of **2** and **3** as determined by ^{1}H NMR; overall recovery 86%) as a white solid from the two combined batches.

To a 2-L, 3-necked flask equipped with a thermocouple, mechanical stirrer and reflux condenser were charged the 1:1 mixture of the acetoxy and hydroxy β-lactams (82 g) and 1720 mL (20 volume) of a 60:40 mixture of heptane/toluene. The mixture was heated to 70–75°C (internal temperature) and held for 4–5 h. The mixture was cooled to room temperature over 4–5 h and stirred for 16–18 h. The reaction mixture was filtered to remove alcohol **3** and then concentrated under reduced pressure; the resulting slightly sticky solids were then dried under high vacuum to give 55 g of predominantly acetoxy β-lactam **2**. This material was charged into a 1-L, 2-necked flask equipped with a magnetic stirrer and nitrogen inlet adapter. Methanol (565 ml) was charged into the reactor followed by anhydrous potassium carbonate (0.42 g; 1 mol%). The reaction mixture

was stirred at room temperature (22–25°C) and monitored by TLC (silica gel; ethyl acetate/hexane 2:1; R_F (**2**) = 0.42; R_F (**3**) = 0.2) for the disappearance of the acetoxy β-lactam. After the reaction was complete the pH of the reaction mixture was adjusted to 7 using an acidic resin (AG 50W×8; 50–100 mesh size, hydrogen form). The reaction mixture was then filtered through a pad of Celite to remove the resin. Methanol was then removed on the rotary evaporator at ambient temperature under reduced pressure. The crude hydroxy β-lactam was then dried in a vacuum oven at 50°C (house vacuum with a flow of nitrogen) to give 39.7 g of material. To a 1-L, 3-necked flask equipped with a magnetic stirrer, temperature probe and reflux condenser was charged the crude hydroxy β-lactam **4** (37.4 g) followed by 561 mL of reagent grade ethyl acetate. The mixture was heated to reflux to dissolve the β-lactam. The reaction mixture was then cooled to 60–65°C and seeded with the enantiomerically pure hydroxy β-lactam **4**. The mixture was stirred at 60–65°C for 4 h during which time the product started to crystallize. The pot temperature was lowered to room temperature over 4–5 h and the mixture was stirred at room temperature (22–24°C) for 16–18 h. The reaction mixture was cooled to 0°C and stirred for 1 h. Filtration followed by washing with 2×50 mL of cold ethyl acetate and drying under reduced pressure at 40–50°C gave 29.7g of **4** as a white solid (87% recovery; >99.9% ee as determined by chiral HPLC). ^{1}H NMR (300 MHz; DMSO-d_6): δ 8.0.92 (s, 9H), 3.20 (d, J = 4.9 Hz, 1H), 4.67–4.70 (m, 1H), 5.91 (d, J = 6.8 Hz, 1H), 8.11 (s, 1H) ppm. ^{13}C NMR (75 MHz; DMSO-d_6): δ 24.3, 30.9, 60.7, 74.7, 169.2 ppm. Anal. calcd for $C_7H_{13}NO_2$: C, 58.72; H, 9.15; N, 9.78. Found: C, 58.72; H, 9.11; N, 9.81. HRMS: calcd for $C_7H_{14}NO_2$: 144.1023 $(M+H)^+$. Found:144.1025 $(M+H)^+$. A similar procedure was used to isolate 60 g of **4** from the 7.5-L reaction mixture obtained using the immobilized BMS lipase.

4.5. Analytical methods

The racemic acetate and racemic alcohol were analyzed by HPLC. A Nova Pak C18 reverse-phase column (4×150 mm) was used. The mobile phase was 15% (v/v) acetonitrile in water, and the flow rate was 1 mL/min. The detection wavelength was 227 nm. The retention times for the acetate and alcohol were 15.2 and 3.2 min, respectively. The separation of enantiomers of acetate and alcohol was carried out by chiral HPLC. A Chiralcel AD column was used (Diacel Chemical Industries Inc. Ltd, Chiral Technologies, Easton, PA). The mobile phase consisted of 1.96% absolute ethanol in hexane and was used at 1 mL/min at ambient temperature. The detection wavelength was 210 nm. The retention times for the two acetate enantiomers were 22 and 50 min, respectively. The retention times for the two alcohol enantiomers were 36 and 40 min, respectively.

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