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A practical enzymatic procedure for the resolution of *N*-substituted 2-azabicyclo[2.2.1]hept-5-en-3-one

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Abstract

A simple and efficient process for the enantioselective resolution of *N*-substituted 2-azabicyclo[2.2.1]hept-5-en-3-one has been developed using commercially available hydrolytic enzymes. This offers a practical approach for the preparation of enantiomerically pure *N*-substituted γ-lactams.

1. Introduction

Abacavir **1** (Ziagen™), a 2-aminopurine nucleoside analogue, is a selective and potent reverse transcriptase inhibitor which is being evaluated at Glaxo Wellcome for the treatment of human immunodeficiency virus (HIV) and hepatitis B virus (HBV) infections in adults and children.[1] An important step in the manufacture of abacavir is the preparation of enantiomerically pure *N*-substituted γ-lactams **2** and **3**, Fig. 1.

We report here on a simple, cost effective and practical enzymatic procedure for the preparation of **2** and **3** in very high enantiomeric excess and chemical yields.

2. Results and discussion

Carbocyclic analogues of purine and pyrimidine nucleosides have generated a great deal of interest as anti-HIV and anti-herpetic agents.[2–4] We at Glaxo Wellcome have spent a number of years investigating the potential of carbocyclic nucleosides such as abacavir **1**, carbovir **5**, carbocyclic 2′-deoxy-

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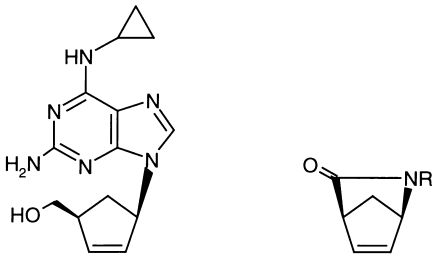
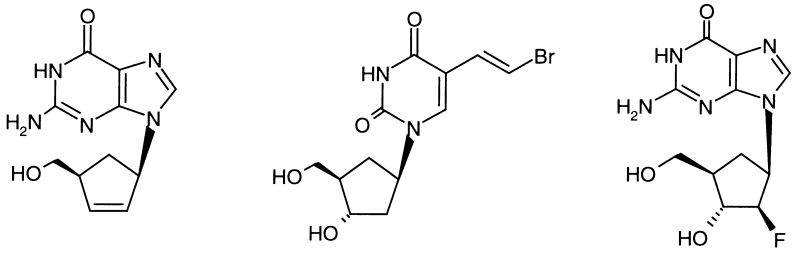


[1]

[2] R= $COOC(CH_3)_3$, [BOC]
[3] R= $COCH_3$, [acetyl]
[4] R= H

Figure 1. Structures of abacavir and *N*-substituted γ-lactam intermediates

5-[(*E*)-2-bromovinyl]uridine **6** (*c*-BVdU) and carbocyclic 2′-*ara*-fluoro-guanosine **7** as antiviral agents (Fig. 2).[4–10]



[5] [6] [7]

Figure 2. Structures of selected carbocyclic nucleosides as antiviral intermediates

The γ-lactam **4** (2-azabicyclo[2.2.1]hept-5-en-3-one), is a useful intermediate which, may be used in the synthesis of these agents. The Exeter and Chiroscience groups have developed a process for the resolution of racemic **4**, using γ-lactamase containing microorganisms such as *Pseudomonas solanacearum* NCIMB 40249 and *Rhodococcus sp*. NCIMB 40213, to give enantiomerically pure (−)-**4** or (+)-**4** and the corresponding amino acid.[11,12] These enzymes are, however, not commercially available for general use.

The *N*-substituted γ-lactam **2** is a key intermediate in the synthesis of abacavir **1**. We, therefore, embarked on a programme to produce **2** in an enantiomerically pure form. It was argued that by activating the lactam ring with acyl protecting groups such as BOC or acetyl (Fig. 1) we may be able to find a conventional hydrolytic enzyme, rather than needing a specialised γ-lactamase, that would hydrolyse the lactam bond of **2** and **3** enantioselectively.

A number of commercially available hydrolytic enzymes (esterases, lipases, proteases), of microbial and mammalian origin, were screened for the ability to hydrolyse the lactam bond of racemic **2** [(±)-*tert*-butyl 3-oxo-2-azabicyclo(2.2.1)hept-5-ene-2-carboxylate] enantioselectively. Reactions were monitored using an on-line HPLC optical rotation detector (chiralyser). There was substantial chemical hydrolysis of the *N*-BOC protecting group under aqueous conditions, but this could be minimised if reactions contained up to 50% (v/v) organic solvents such as tetrahydrofuran.

Indeed, we found that a number of these enzymes hydrolysed (+)-(1*S*,4*R*)-**2** to the corresponding *N*-acyl amino acid leaving behind the residual (−)-(1*R*,4*S*)-**2** of the correct absolute configuration for synthesis of abacavir (Fig. 3).

These enzymes were pig liver esterase (ALTUS), *Bacillus* sp. protease (ALTUS), *Subtilisin carlsberg* (ALTUS), Neutrase (*Bacillus subtilis*, NOVO), Novozyme 243 (*Bacillus licheniformis*, NOVO), Alca-

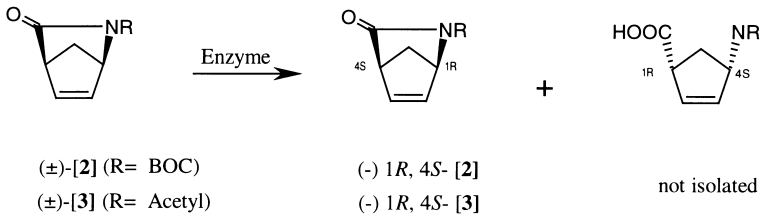


Figure 3. Enzymatic resolution of *N*-substituted lactams

lase (*Bacillus licheniformis*, NOVO), Savinase (*Bacillus* sp. NOVO), porcine pancreatic lipase (Biocatalysts), Flavorpro-192 (peptidase, Biocatalysts), Flavorpro-373 (glutaminase, Biocatalysts), Promod-TP (endopeptiadse, Biocatalysts), lipase-CE (*Humicola lanuginosa*, Amano), protease-M (*Aspergillus* sp., Amano), prozyme-6 (*Aspergillus* sp., Amano), lipase PGE (calf tongue root and salivary gland, Amano) and *Aspergillus* sp. acylase (Sigma).

Savinase was selected for further investigation. Savinase (Subtilisin, EC 3.4.21.62) is a serine-type protease, which is produced by submerged fermentation of a genetically modified alkalophilic *Bacillus* sp. This enzyme is inexpensive and available in bulk for use in the detergent industry.

Savinase was found to be highly enantioselective. Typically, reactions were carried out at 30°C in phosphate buffer (pH 8.0) containing up to 50% (v/v) organic solvent such as tetrahydrofuran and up to 100 g/l of racemic **2**. Reaction mixtures were monitored by reverse-phase and chiral HPLC. Upon completion of reactions (50% conversion) the enantiomeric excess of (−)-**2** was better than 99%. The reaction mixture was filtered and (−)-**2** was isolated in good chemical yield (84% theory) by extraction into cyclohexane followed by evaporation of the organic solvent.

Similarly, Savinase enantioselectively hydrolysed the lactam bond of racemic **3** (*cis*-2-acetyl-2-azabicyclo[2.2.1]hept-5-en-3-one) to afford (−)-(1*R*,4*S*)-**3** (Fig. 3). The reaction mixture, containing ca. 50% of the starting material, was analysed by chiral HPLC and the residual lactam was shown to have an ee >99% with the correct absolute configuration for synthesis of abacavir. Interestingly, Savinase did not hydrolyse the unactivated racemic **4**.

In conclusion, we have found that by activating the γ-lactam ring of **4**, and by protecting the nitrogen atom with groups such as BOC or acetyl,[13] we were able to hydrolyse enantioselectively *N*-substituted γ-lactams using commercially available hydrolytic enzymes rather than specialised γ-lactamases. This has formed the basis for development of a simple and scaleable process for preparation of enantiomerically pure *N*-substituted γ-lactams.

3. Experimental

3.1. General procedures

Enzymes used were obtained from Altus, Novo, Biocatalysts Ltd, Amano, Sigma Chemical Co. and Boehringer Mannheim. The enzyme units are as defined by these suppliers. All chemicals used were of AR grade (BDH or Fissons). Analytical reagent or HPLC grade solvents were used throughout (Rathburn Chemicals Ltd).

Melting points were determined on an Electrothermal Digital Melting Point Apparatus. Spectra were recorded using the following instruments: ^{1}H NMR Bruker DPX 250 MHz; mass spectra with a Micromass Platform, LC/MS, +ve ion scans, Electrospray; infra red spectra with a Nicolet 20SXC FTIR spectrometer. Solvents were evaporated on a rotary evaporator.

3.2. Analytical methods

The extent of bioconversions were determined using reverse-phase HPLC or chiral HPLC. This consisted of a Hewlett–Packard HPLC system equipped with a diode array detector and an on-line Chiralyser (IBZ, Messtechnik, Germany) connected in series. The reverse-phase system was also used to monitor product isolation. Prior to analysis, samples were filtered (Gelman, Acrodisc-LC, 0.45 μ), the filtrate was diluted 1:2 in water and 50 μl was injected onto the reverse-phase column. For chiral analyses, samples were dried under vacuum and the residue was dissolved in 20 μl of 2-propanol or ethanol and injected onto the chiral column.

3.3. HPLC analysis of **2**

Reverse-phase HPLC: column, Spherisorb C6 (15×0.46 cm); mobile phase, 30% (v/v) acetonitrile/0.1% (v/v) formic acid; flow rate, 1 ml/min; detection wavelength, 200 nm; temperature, 20°C. Chiral HPLC: column, Chiralcel OD-H (25×0.46 cm); mobile phase, 2% (v/v) 2-propanol/heptane; flow rate, 0.5 ml/min; detection wavelength, 205 nm; temperature, 5°C.

3.4. HPLC analysis of **3**

Reverse-phase HPLC: column, Spherisorb C6 (15×0.46 cm); mobile phase, 5% (v/v) acetonitrile/0.1% (v/v) formic acid; flow rate, 1 ml/min; detection wavelength, 210 nm; temperature, 20°C. Chiral HPLC: column, Chiralpak AD (25×0.46 cm); mobile phase, 2% (v/v) ethanol/98% (v/v) heptane; flow rate, 1 ml/min; detection wavelength, 215 nm; temperature, 20°C.

3.5. Synthesis of racemic **2** [(±)-tert-butyl 3-oxo-2-azabicyclo(2.2.1)hept-5-ene-2-carboxylate]

A solution of di-*tert*-butyl dicarbonate (110.0 g) in tetrahydrofuran (50 ml) was added slowly to a suspension of racemic **4** (50.0 g), and 4-dimethylaminopyridine (0.5 g) in tetrahydrofuran (150 ml). The brown, hazy solution was stirred at 20°C until reaction was complete by TLC (SiO_2, CH_2Cl_2:MeOH, 96:4; starting material R_f=0.28, product R_f=0.76, detection UV and alkaline permanganate spray). The solution was concentrated in vacuo to give a brown foam. Recrystallisation twice from cyclohexane afforded the product (racemic **2**) as pale pink crystals (67.8 g, 70.7% theory). Mp 70.5–71.5°C; IR (Nujol) 1752 cm^{-1} (C=O), 1709 cm^{-1} (NC=O); ^1H NMR ($CDCl_3$): δ 1.5 (s, 5H), 2.15 (d, 1H, *J* 8.5 Hz), 2.35 (d, 1H, *J* 8.5 Hz), 3.39 (s, 1H), 4.96 (s, 1H), 6.66 (m, 1H), 6.89 (dd, 1H, *J* 5.6, 2.1 Hz); MS: MH^+ 210.

3.6. Synthesis of racemic **3** [(±) cis-2-acetyl-2-aza-bicyclo[2.2.1]hept-5-en-3-one]

Acetic anhydride (38.0 ml) was added dropwise to a solution of racemic **4** (20.0 g), and triethylamine (56.2 ml) in tetrahydrofuran (80 ml). The colourless solution was heated to reflux and stirred for 23 h. Charcoal (5.0 g) was added, the mixture was stirred for 20 min and then filtered. The solution was concentrated in vacuo to remove the tetrahydrofuran and the residue was partitioned between water (100 ml) and cyclohexane (3×100 ml and 12×50 ml). The combined organic extracts were backwashed with water (50 ml) and brine (50 ml) and then dried over magnesium sulphate. Water (30 ml) was added and the reaction mixture was extracted with ethyl acetate (3×20 ml). The dark brown organic layer was treated with charcoal (1.0 g) and stirred for 15 min. The mixture was filtered and evaporated to yield

racemic **3** as a light brown oil (5.03 g, 74.0% theory). IR (film) 1749 cm^{-1} (C=O), 1690 cm^{-1} (NC=O); ^1H NMR ($CDCl_3$): δ 2.21 (d, 1H, 8.6 Hz), 2.32 (d, 1H, 8.6 Hz), 2.38 (s, 3H), 3.45 (s, 1H), 5.29 (s, 1H), 6.67 (m, 1H), 6.90 (dd, 1H, *J* 5.0, 2.0 Hz); MS: MH^+ 152.

*3.7. Preparation of (−)-**2** [(1*R*,4*S*)-tert-butyl 3-oxo-2-azabicyclo(2.2.1)hept-5-ene-2-carboxylate] by enzymatic resolution*

Savinase (30 g) was added to a solution (500 ml) containing 10 g of (±)-**2** in 50% tetrahydrofuran: 50% phosphate buffer (50 mM, pH 8.0) at 30°C. The reaction was monitored by HPLC for up to 2 days. Upon completion of the reaction (51% conversion), the enzyme was filtered and the pH of the clarified solution was raised to 9 with a sodium bicarbonate solution. This was then extracted with 3×200 ml of cyclohexane. The combined organic phase was back extracted with 100 ml of sodium bicarbonate solution and subsequently washed with 100 ml of brine. Evaporation and drying yielded a free flowing white solid (−)-**2** (4.2 g, 84% theory isolated yield) which was identified by ^1H NMR and by co-injections and spiking with an authentic enantiomerically pure standard. The enantiomeric excess was better than 99% as analysed by chiral HPLC. Mp 88.6°C; IR (Nujol) 1752 cm^{-1} (C=O), 1709 cm^{-1} (NC=O); ^1H NMR ($CDCl_3$): δ 1.5 (s, 5H), 2.15 (d, 1H, *J* 8.5 Hz), 2.35 (d, 1H, *J* 8.5 Hz), 3.39 (s, 1H), 4.96 (s, 1H), 6.66 (m, 1H), 6.89 (dd, 1H, *J* 5.6, 2.1 Hz); MS: MH^+ 210.

*3.8. Preparation of (−)-**3** [(1*R*,4*S*)-2-acetyl-2-aza-bicyclo[2.2.1]hept-5-en-3-one] by enzymatic resolution*

The reaction was carried out on an analytical scale in a magnetically stirred glass vial (4 ml working volume) containing 1 mg/ml of (±)-**3** in 50% tetrahydrofuran: 50% phosphate buffer (50 mM, pH 7.0) at room temperature. The reaction was started by adding Savinase to a final concentration of 25 mg/ml. A flask with no enzyme served as control. Periodically, samples were removed and diluted 1:2 with water prior to HPLC analyses. It was shown that, in the absence of an enzyme, chemical hydrolysis of the substrate was negligible under the reaction conditions. Savinase hydrolysed (±)-**3** enantioselectively to afford (−)-(1*R*,4*S*)-**3** as evidenced by a negative sign of rotation by chiralyser and by chiral HPLC analysis. The identity of (−)-**3** was confirmed by co-injections and spiking with an authentic enantiomerically pure standard. The reaction mixture, containing ca. 50% of the starting material, was analysed by chiral HPLC and the residual lactam was shown to have an ee >99%.

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