
Whole Cell Catalysed Kinetic Resolution of 6-Azabicyclo[3.2.0]hept-3-en-7-one: Synthesis of (–)-Cispentacin (FR 109615)

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Enantioselective hydrolysis of the β -lactam (\pm)-**2** using *Rhodococcus equi* provided (1*R*,5*S*)-6-azabicyclo[3.2.0]hept-3-en-7-one (+)-**2**, a precursor of the antifungal agent cispentacin.

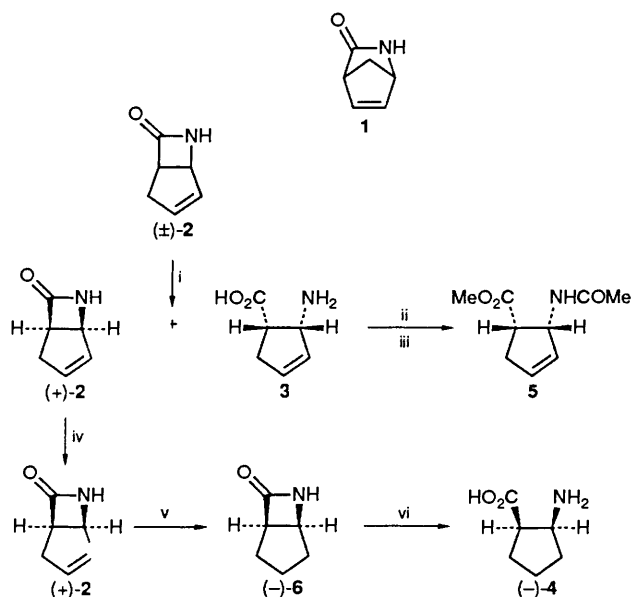
Our recent work on the biocatalytic resolution of the carbocyclic nucleoside precursor (\pm)-2-azabicyclo[2.2.1]hept-5-en-3-one **1**¹ prompted us to investigate a similar strategy for resolution of the isomeric β -lactam (\pm)-6-azabicyclo[3.2.0]hept-3-en-7-one **2**.² Our interest was heightened by the obvious potential of **2** (or the corresponding amino acid **3**) to provide a precursor of the antifungal antibiotic cispentacin³ (FR 109615) **4**.^{4,5} Herein we report the result of these investigations.

The resolution of (\pm)-**2** with commercially available isolated enzymes proved unsuccessful. Thus porcine kidney aminoacylase catalysed a non-specific hydrolysis of the lactam while *Aspergillus* sp. aminoacylase, β -lactamases from *Bacillus cereus*, *Enterobacter cloacae*, *Escherichia coli* or *Staphylococcus aureus*, bovine pancreatic α -chymotrypsin, porcine pancreatic trypsin, proteases from *Bacillus subtilis*, *Bacillus thermoproteolyticus*

roko or *Aspergillus oryzae*, and porcine pancreatic lipase gave little or no hydrolysis.

Resolution was, however, achieved in a highly selective manner using a whole cell preparation ENZA-1 (*Rhodococcus equi* NCIB 40213), an organism which had previously been utilized in the resolution of **1**. Thus, incubation of the cells at 20 °C in water buffered to pH 7 with the lactam until *ca.* 45% hydrolysis occurred gave amino acid **3** which was converted without purification into the corresponding methyl ester acetamide **5** (38% yield based on racemic lactam) (Scheme 1). This material was shown to have an enantiomeric excess (ee) of 96% by gas chromatographic analysis on a Lipodex-D column.

The recovered lactam was then reincubated under the same conditions until the ee was >99%, as assessed by GC analysis, giving a 40% recovery (based on racemic starting material) of



Scheme 1 Reagents and conditions: i, ENZA-1, pH 7, 20 °C [(+)-2, 53%, 75% ee]; ii, (MeO)₂CMe₂, MeOH, HCl; iii, Ac₂O, pyridine, CH₂Cl₂ [38%, from (±)-2; 96% ee]; iv, ENZA-1, pH 7, 20 °C [40% from (±)-2; >99% ee]; v, H₂, Pd/C, EtOAc (95%); vi, HCl, H₂O (95%)

(+)-2. The lactam (+)-2 was hydrogenated to give the fully saturated analogue (-)-6 which was, in turn, hydrolysed to give the corresponding amino acid (-)-4. This material displayed a similar optical rotation $[\alpha]_D -8$ (*c* 1, H₂O)† to that reported for natural cispentacin, $[\alpha]_D -10.7^3$ and $-8.9^{4,5}$ (*c* 1, H₂O), which is reported to have 1*R*,2*S*-stereochemistry.⁵ On this basis we can assign 1*R*,2*S*-stereochemistry to the lactams (+)-2 and (-)-6.

It is interesting to note that ENZA-1 shows poor hydrolytic activity towards (±)-6.

Experimental

Enantioselective Hydrolysis of the Lactam 2.—*Rhodococcus*

† Units for $[\alpha]_D$ expressed in 10⁻¹ deg cm² g⁻¹.

equi NCIB 40213 (700 mg of paste) was suspended in phosphate buffer (0.05 mol dm⁻³; pH 7) and 6-azabicyclo[3.2.0]hept-3-en-7-one 2 (340 mg, 3.12 mmol) was added. Stirring was continued at room temperature for 142 h after which the cells were removed by centrifugation. The supernatant was extracted with dichloromethane (4 × 100 ml) and the combined organic layers were dried (MgSO₄) and concentrated. The recovered lactam (197 mg) was reincubated with *Rhodococcus equi* (280 mg) in buffer (36 ml) for a further 170 h and then recovered as above. Column chromatography over silica using ethyl acetate as eluent gave (+)-6-azabicyclo[3.2.0]hept-3-en-7-one (+)-2 (137 mg, 40%) as a white solid; m.p. 76–77 °C, $[\alpha]_D^{20}$ (*c* 0.4, CHCl₃) 37; $\nu_{\max}/\text{cm}^{-1}$ (CHCl₃) 3414 and 1754; $\delta_{\text{H}}(\text{CDCl}_3)$ 6.25 (1 H, br s, NH), 6.12–5.85 (2 H, m, 3-H and 4-H), 4.59–4.42 (1 H, m, 5-H), 3.85 (1 H, ddd, *J* 9.8, 3.5, 3.5, 1-H) and 2.90–2.25 (2 H, m, 2-H₂).

Acknowledgements

We thank Carol Lee (Enzymatix Ltd) for a supply of microorganisms, Enzymatix for a Lectureship and Enzymatix, the SERC and the DTI for a postdoctoral Fellowship under the aegis of the Biotransformation LINK Scheme (both to A. G. S.).

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Paper 1/02852I

Received 13th June 1991

Accepted 25th June 1991