Chemoenzymatic Synthesis of (−)-Carbovir utilizing a Whole Cell Catalysed Resolution of 2-Azabicyclo[2.2.1]hept-5-en-3-one

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The resolution of (±)-2-azabicyclo[2.2.1]hept-5-en-3-one (**3**), a versatile intermediate in the synthesis of carbocyclic nucleosides, is described; both optical forms of the lactam have been obtained in very high optical purity (>98% enantiomeric excess) in rapid, facile, large-scale biotransformation processes using whole cell catalysts and the laevorotatory enantiomer has been converted into (−)-carbovir.

Carbocyclic analogues of purine and pyrimidine nucleosides, in which a methylene group replaces the oxygen atom of the (deoxy)-ribofuranose ring have generated great interest as potential anti-viral agents in recent years.[1] Furthermore, the dideoxydidehydrocarbocyclic nucleoside, (±)-carbovir, has proven to be a potent and selective inhibitor of HIV-1 *in vitro*.[2] Indeed, its hydrolytic stability and ability to inhibit infectivity and replication of the virus in human T-cells lines at concentrations 200—400 fold below toxic levels make carbovir a promising candidate for development as a potential anti-retroviral agent.

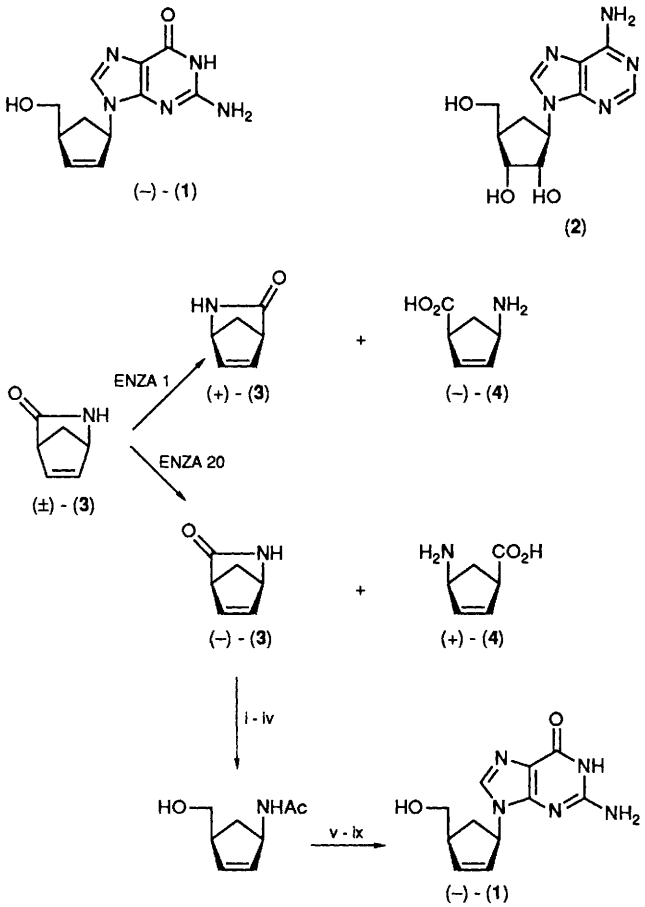
Studies undertaken at the laboratories of Glaxo Group Research have resulted in the characterization of optically pure (−)-carbovir, (−)-(**1**), *via* a synthetic route beginning with the chiral natural product aristeromycin (**2**).[3] Subsequent biological evaluation of (−)-carbovir in whole cell assays using MT-4 cells demonstrated that (−)-carbovir has similar activity against HIV (RF strain) to AZT (zidovudine) ($I.C._{50}$ 0.0015 μg ml^{-1} and 0.001 μg ml^{-1} respectively).

Whilst this work demonstrated for the first time the efficacy of (−)-carbovir, *in vitro*, the synthetic route has two limitations. First aristeromycin, the requisite starting material, is not readily available. Secondly the Glaxo route gives access to only one enantiomer of the carbocyclic nucleoside. In view of the recent findings of Griengl and de Clercq[4] that both enantiomers of some carbocyclic nucleosides can display potent biological activity, a more versatile strategy that could furnish both enantiomers of carbovir was required urgently.

A synthesis of racemic carbovir has been described by Vince and Hua.[5] The key intermediate in this route is (±)-2-azabicyclo[2.2.1]hept-5-en-3-one (**3**) which is produced by addition of tosyl cyanide to cyclopentadiene followed by acid work-up. Herein we describe the use of two distinct whole-cell biocatalysts in the kinetic resolution of the racemic azanorbornenone (**3**) to generate both enantiomers of this lactam showing very high optical purities [>98% enantiomeric excess (e.e.)].

Thus enantiospecific and enantiocomplementary hydrolyses of (±)-2-azabicyclo[2.2.1]hept-5-en-3-one (**3**) were catalysed by whole cell preparations of microbial strains ENZA-1 and ENZA-20 (Scheme 1). Both strains were isolated from the environment under conditions designed to select for organisms capable of growth on a range of *N*-acyl compounds as the sole source of carbon and energy. Subsequent to isolation, ENZA-1 (*Rhodococcus equi* NCIB 40213) and ENZA-20 (*Pseudomonas solanacearum* NCIB 40249) were grown up separately to very high biomass levels (>40 g l^{-1}) in liquid culture (24—48 h) and the resulting cells were harvested by centrifugation. Cells of each organism were incubated at 25 °C in water buffered to pH 7 in the presence of the lactam; the reduction in lactam concentration was followed by means of UV spectroscopy and reverse phase HPLC. The reaction was quenched at 55% conversion by harvesting the cells and the lactam remaining in the aqueous phase was isolated by extraction into dichloromethane. In a typical run, biocatalyst concentration was 6 g dry weight l^{-1} and the initial concentration of the lactam was 50 g l^{-1}; the bioconversion was complete in 3 h. The low concentration of enzyme, high concentration of the substrate, and the speed of the biotransformation are noteworthy. Moreover, mutant strains of ENZA-1 and ENZA-20 have been constructed which hyperexpress the γ-lactamase activity. The use of these mutants as whole cell biocatalysts enables extremely rapid rates of catalysis to be carried out in the presence of very high substrate concentrations, >100 g l^{-1}.

The optical purity of the recovered lactam was assessed by NMR spectroscopy using a chiral shift reagent. From the fermentation using the microorganism ENZA-1, (+)-lactam was obtained, $[\alpha]_D^{25}$ +555° ± 15° (>98% e.e., 45% yield). From an equally facile bioconversion ENZA-20 produced the (−)-lactam, $[\alpha]_D^{25}$ −568° ± 15° (>98% e.e., 45% yield). After removal of the lactam the corresponding optically active amino acid (+)- or (−)-(**4**) can be recovered from the aqueous phase as the hydrochloride by acidification (HCl) and



Scheme 1. *Reagents and conditions*: i, HCl, H_2O, reflux; ii, $(MeO)_2CMe_2$, MeOH, HCl; iii, Ac_2O, pyridine, CH_2Cl_2 (96%); iv, $Ca(BH_4)_2$, tetrahydrofuran, ultrasound (73%); v, HCl, H_2O, EtOH, reflux; vi, 2-amino-4,6-dichloropyrimidine, Pr^i_2NEt, Bu^nOH, reflux (80%); vii, 4-$ClC_6H_4N_2^+Cl^-$, HOAc, NaOAc, H_2O (69%); viii, Zn, AcOH, EtOH, H_2O, reflux (50%); ix, $(EtO)_3CH$, HCl then HCl, H_2O (92%); x, NaOH, H_2O, reflux (74%).

evaporation of the water. The laevorotatory lactam (−)-(**3**) was converted into (−)-carbovir (**1**) by a series of reactions (Scheme 1) under conditions designed to improve the reliability of the published route.[5] Note that the optically active lactams (+)- and (−)-(**3**) can be converted into a wide range of interesting homochiral compounds.[6] Furthermore the amino-acids (+)- and (−)-(**4**) are also available from this simple biotransformation at high optical purity, a strategy for their preparation that is more convenient than the previously recorded method using an esterase.[7]

The versatility of the micro-organisms ENZA-1 and ENZA-20 for the stereoselective transformation of other lactams is under investigation.

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