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Enzymic Resolution of Oxathiolane Intermediates - An Alternative Approach to the Anti-viral Agent Lamivudine (3TC™†)

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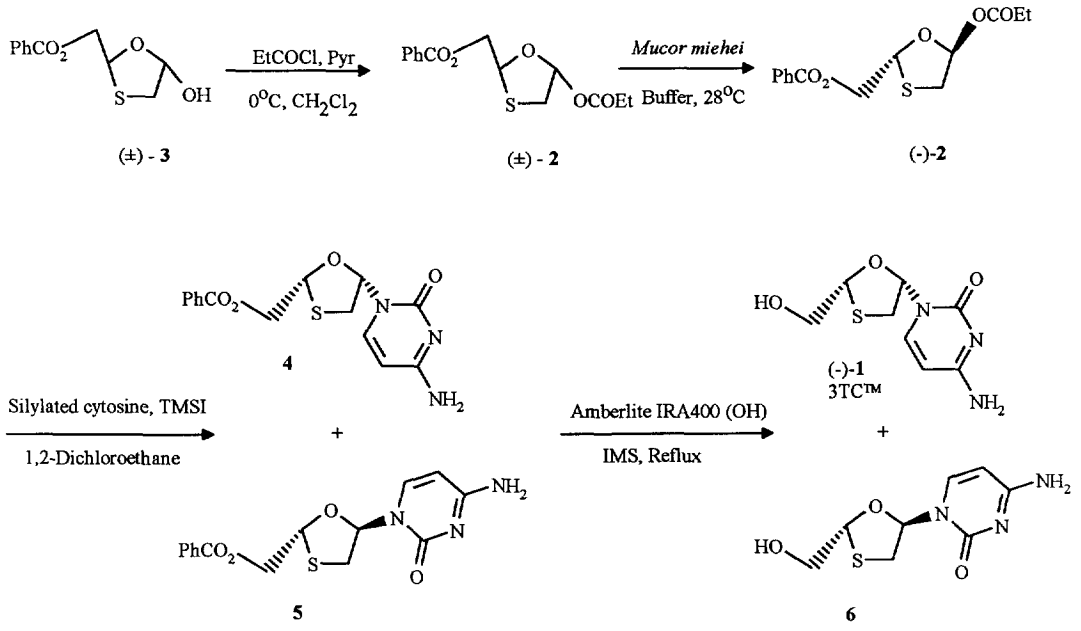
Abstract: A number of commercially available lipases and proteases was screened for the ability to hydrolyse enantioselectively racemic oxathiolane **2**. *Mucor miehei* lipase was identified as the most efficient biocatalyst. Bioconversion of **2** afforded enantiomerically-enriched residual ester of the correct absolute stereochemistry, (-)-2*R*, for subsequent synthesis of the anti-viral agent lamivudine, (3TC™).

The nucleoside analogue 4-amino-1-(hydroxymethyl-[1,3]oxathiolanyl)-1H-pyrimidin-2-one (BCH189), (±)-**1**, displays very interesting biological activity since both enantiomers inhibit human imunodeficiency virus types 1 and 2 *in vitro*.[1] However, the 'unnatural' (-)-**1** enantiomer (3TC™) is considerably less toxic than the corresponding 'natural' (+)-enantiomer and is currently undergoing clinical evaluation.[2] Enantiomerically pure (+)-**1** has been synthesised from both D-mannose[3] and D-galactose,[4] whereas 3TC™ has been synthesised from L-gulose[5] and from (+)- thiolactic acid in four steps.[6] A large scale resolution process for 3TC™ has been developed at Glaxo Research and Development Ltd.[7] It utilises immobilised cytidine deaminase from a recombinant strain of *Escherichia coli* to deaminate racemic **1** enantioselectively, leaving behind essentially enantiomerically pure 3TC™. Enzymes have been used frequently in the synthesis of nucleosides. Notably, carbocyclic nucleosides have been resolved by enantiospecific hydrolysis of their monophosphates[8] and the 5-fluoro derivative of BCH189 has been resolved by enantioselective hydrolysis of the corresponding butyrate ester.[9] We have recently described a systematic survey of the resolution of ester intermediates, prior to base addition, for the synthesis of carbocyclic nucleosides.[10] We report here on a similar approach to resolve racemic oxathiolane propionate **2**, to obtain the key intermediate (-)-**2** in good enantiomeric excess, for the synthesis of 3TC™.

As an alternative to asymmetric total synthesis of 3TC™ and to avoid late stage resolution of racemic **1**, we chose to investigate enzymic methods to resolve intermediates prior to the addition of the cytosine base (**Scheme 1**). The most pertinent substrates for this approach were the oxathiolane benzoates **2** and **3**, as these substrates could be easily accommodated into the existing synthetic route. Our attention was focused on the *trans*-oxathiolane benzoate **2** where we envisaged production of enantiomerically-enriched (-)-**2** by enantioselective hydrolysis of the anomeric alkyl ester; the hydrolysed hydroxyoxathiolane would be unstable to the reaction conditions thus aiding the isolation and purification of the desired product (i.e. the residual substrate). Racemic **2** was easily prepared in a good yield (86%) from hydroxyoxathiolane **3** which had been obtained from Process Research Department, Glaxo Research and Development Ltd.[11], (**Scheme 1**). The acylation of **3** proceeds stereospecifically to give the *trans*-propionate **2** which contained only 2% of the *cis*-isomer as evidenced by ^{1}H nmr and hplc.

† 3TC™ is a registered trade mark of the Glaxo group of companies.



Scheme 1

Nine lipases and two proteases were screened for their ability to hydrolyse enantioselectively racemic **2** (**Table 1**).[12] The reactions were carried out in magnetically-stirred glass vials (2-5 ml working volume), with 40-300 units of the biocatalyst per ml at 20°C, and the enantiomeric excess (ee) of the residual substrate was analysed by chiral hplc. Amongst the eleven enzymes screened, the highest enantioselectivity (E, 11) was obtained with *Mucor miehei* lipase. All other enzymes tested were less enantioselective than *Mucor miehei* lipase, but it is interesting to note that *Candida cylindracae* and *Chromobacterium viscosum* lipases and subtilisin showed the opposite enantioselectivity to *Mucor miehei* lipase albeit in very low ee.

In order to determine the absolute configuration of the residual ester, enough material was required to synthesise the final nucleoside product for comparison with authentic material. The *Mucor miehei* lipase was selected for the scale up of the reaction with **2** and a small batch of enzyme (300 units) was used to process 500 mg of **2** at 1 mg/ml. The concentration of biocatalyst was much less than that used in the small scale screen and therefore required a far longer incubation time. After 3 weeks of incubation at 28°C, 75% of **2** had been hydrolysed (ee, 70-80%) as evidenced by hplc. At harvest and after purification, optically active (-)-2*R*-*trans*-oxathiolane propionate (-)-**2** ($[\alpha]_D^{22}$ -28.5; c 0.65, CH_2Cl_2) was obtained in 14% yield (28% theory yield). An ee of 76% for this material was determined by chiral hplc and 80% by chiral shift proton nmr using (-)-*R*-2,2,2-trifluoro-1-(9-anthryl)ethanol as chiral solvating agent.

Table 1. Enzyme Catalysed Resolution of Racemic **2**

Biocatalyst	Time (h)	Conversion (%)	Residual ester[++] (%ee)	Absolute configuration[†]	E [♣]
Aspergillus niger lipase [1]	1.5	67	16	(-)-2*R*	1
Candida cylindracae lipase [2]	3	89	5	(+)-2*S*	1
Chromobacterium viscosum lipase [1]	15	91	10	(+)-2*S*	1
Lipase B1 [3]	3	87	88	(+)-2*R*	3
Lipase F13 [3]	24	67	48	(-)-2*R*	2
Lipase F14 [3]	5	44	39	(-)-2*R*	4
Mucor miehei lipase [1]	2	48	65	(-)-2*R*	11
Pseudomonas fluorescens lipase [1]	15	71	63	(-)-2*R*	3
Rhizopus delemar lipase [1]	22	34	36	(-)-2*R*	8
Subtilisin [2]	0.01	34	15	(+)-2*S*	2
Trypsin [2]	5	34	0	(±)	-

[++], ee (enantiomeric excess) by chiral hplc; [†], all compounds in *trans* series; [♣], E (enantiomeric ratio, see ref. 13); Suppliers: 1, Biocatalysts Ltd.; 2, Sigma; 3, Chiroscience Ltd..

The addition of the cytosine base to (-)-**2** was given careful consideration since the two newly formed stereogenic centres could potentially racemise under the Lewis acid conditions routinely used for the coupling. Coupling of the racemic oxathiolane propionate **2** with silylated cytosine using $SnCl_4$ as the Lewis acid had furnished a favourable mixture of anomers, (*cis:trans* 8:1) in 50% yield. However, racemisation of optically active substrate under these conditions has been described[5] and so negated the use of $SnCl_4$ on (-)-**2**. The enantiomerically-enriched oxathiolane propionate (-)-**2** was coupled with silylated cytosine using trimethylsilyliodide (TMSI) as the Lewis acid to give a diastereomeric mixture of the protected nucleosides **4** and **5** in 50% yield (**Scheme 1**) with a *cis:trans* ratio of 1.3:1, as had been previously observed for this methodology.[6] The chiral hplc of the protected nucleosides proved capricious and so the enantiomerically-enriched material was deprotected under standard conditions using a basic resin in methanol to afford a diastereomeric mixture of nucleosides **1** and **6** in 59% yield after purification with a *cis:trans* ratio of 1.3:1. Chiral hplc of this mixture did not allow separation of the *trans*-diastereoisomer **6** but detected an ee for the *cis*-diastereoisomer **1** of 70%. The major enantiomer was observed to be 3TC™ by comparison with authentic material.

In summary, enzymic resolution of the oxathiolane propionate **2** with *Mucor miehei* lipase affords enantiomerically-enriched residual substrate (-)-**2** which was converted into the nucleoside 3TC™ in good enantiomeric excess (70%). The yields and reaction conditions reported have not been optimised but, nonetheless, this work demonstrates an alternative enzymic route to this potent anti-viral agent where resolution has been applied at a much earlier stage than has previously been reported.

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12. *Enzymic hydrolysis of oxathiolane propionate*, (±)-**2**. All enzymes used were obtained from Sigma Chemical Co., Biocatalysts Ltd. or Chiroscience Ltd.. The enzyme units are as defined by these suppliers. Small scale reactions were carried out in magnetically-stirred glass vials (2-5 ml working volume). The reaction mixtures, containing 1 mg/ml substrate in 100mM Tris-HCl buffer (pH 8) with 2mM $CaCl_2.2H_2O$ and acetonitrile (40% v/v), were incubated at either 20° or 37°C. The reaction was started by adding the enzyme (40-300 units/ml). At intervals, 50µl samples were removed for analysis. Reaction mixtures with no substrate and/or enzymes served as controls. Larger scale conversions (500 mg in 500ml) were carried out at 28°C under similar conditions in magnetically-stirred 2L flasks. In some experiments, reaction mixtures were analysed directly by hplc. When it was necessary to stop the reaction, this was done by ultrafiltration (using Ultrafree Millipore membranes, 10 KD cut-off) followed by centrifugation (10,000g for 15 min.). Control experiments showed that this procedure was effective in stopping the reaction. Reactions were monitored by hplc using the following conditions. Reverse-phase hplc: column, Spherisorb ODS2 (150 x 4.6 mm); mobile phase, 50% (v/v) acetonitrile in 50mM NH_4OAc; flow rate, 2 ml/min; detection wavelength, 232nm. Chiral hplc: column, Microcrystalline cellulose acetate (CONBRIOTAC) (250 x 5 mm); mobile phase, 95% (v/v) ethanol; flow rate, 0.5 ml/min; detection wavelength, 240nm; column temperature, 50°C.
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