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Enzymatic Resolution of α-Acetoxysulfides: A New Approach to the Synthesis of Homochiral S,O-Acetals.

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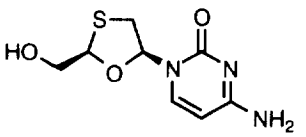
Abstract: A novel enzymatic resolution of α-acetoxysulfides using *Pseudomonas fluorescens* lipase is reported. Selectivity is highly dependent on the substrate and solvent, with enantiomeric excesses of >95% in some cases. We believe these are the first examples of enzymatic resolutions of an S,O-acetal.

The preparation of homochiral secondary acetates by enzymatic resolution is a well established procedure, often giving very high selectivities with appropriate substrates.[1] Our interest in reactive organosulfur intermediates[2] led us to embark on a programme to develop an efficient synthetic route to Lamivudine (3TC™†, **1**), a highly promising drug candidate for HIV[3] and HBV infections.[4] Because of the different toxicities of the two enantiomers of this compound we required a highly enantioselective route and ideally one that would be suitable for large scale synthesis.



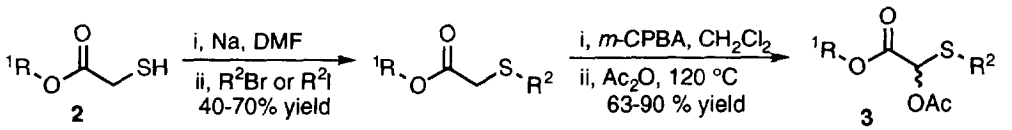
Lamivudine **1**

The enantioselective synthesis of lamivudine **1** provides a considerable challenge to the synthetic chemist due to the presence of the S,O-acetal and aminal stereogenic centres, both sharing the same oxygen atom.[5] Although a number of synthetic routes to racemic S,O-acetals are available[6], to date only those derived from 7-thiomenthol originally reported by Eliel[7], and the asymmetric Pummerer reaction[8], address the problem of control of absolute stereochemistry, of which only the latter was suitable for use in an approach to lamivudine. Despite the particularly encouraging work recently reported by Kita *et al.*[8a-c], our systems gave only minimal chirality transfer from the sulfoxide, and this approach was also limited due to problems accessing the required homochiral sulfoxide precursor.[9] We thus needed an alternative strategy for the

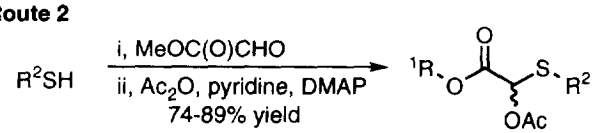
preparation of homochiral α-acetoxysulfides, which led us to consider enzymatic methods. A literature search failed to reveal any examples of enzymatic resolutions of S,O-stereogenic centres.[1] We thus embarked on a systematic study of this interesting and potentially very useful reaction.

The α-acetoxysulfide substrates **3** could be prepared by two routes (scheme 1). Coupling of a mercapto acetate[10] **2** with an alkyl halide, followed by oxidation to the sulfoxide and Pummerer rearrangement (route 1) generally worked well. Alternatively, addition of a thiol[10] to methyl glyoxalate[11] and *in situ* acetylation of the intermediate hemiacetal (route 2) provided a shorter, more convenient procedure.

Route 1



Route 2

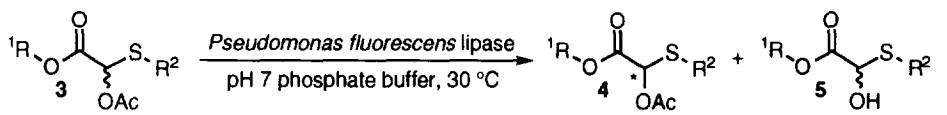


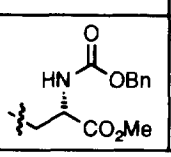
Scheme 1.

With these substrates in hand, we then began to investigate the enzymatic hydrolysis reaction. We initially chose *Pseudomonas fluorescens* lipase because of its reported efficiency for the resolution of more conventional substrates.[1] We were very pleased to observe that the presence of the sulfur atom made no difference to the efficiency of the reaction (table 1). In all cases, exclusive hydrolysis of the acetate group was observed with no detectable hydrolysis of the other ester functionalities. Although the hemithioacetal **5** byproduct could clearly be seen in crude NMR spectra, on purification using column chromatography, only the resolved acetate and thiol (from decomposition of **5**) could be isolated.[12]

The stereoselectivity of the hydrolysis can be rationalised as being controlled by the different sizes of substitutents either side of the acetate chiral centre.[13] With menthyl as the large group (table 1, entries 1-3), an increase in size of the smaller group reduces the selectivity in the hydrolysis from >95% to <10%. Changing to the methyl ester ($R^1 = CH_3$) allows high selectivity to be observed with large groups in R^2, with corresponding reversal of absolute stereochemistry at the acetal chiral centre. It would appear, at least in $CHCl_3$, that the borderline for this change in selectivity is between the dimethyl and diethyl acetals (entries 4 and 6, note optical rotation). However in ^{t}BuOMe solvent no reversal in selectivity is observed between these two substrates. Note also that some particularly lipophilic substrates were essentially inert under the reaction conditions in $CHCl_3$ (entries 8 and 10), but switching to ^{t}BuOMe as solvent restored reactivity (entries 8 *cf.* 9; 10 *cf.* 11), and gave significantly enhanced enantioselectivities (entries 6 *cf.* 7; 12 *cf.* 13; 14 *cf.* 15).[14] Interestingly, in extreme cases, selectivity can begin to decrease if the large group is made too big (entry 9).

In conclusion, we have demonstrated the first enzymatic resolution of an α-acetoxysulfide achieving very high levels of stereocontrol in many cases, and good yields. We have now used this new chemistry to complete the synthesis of **1** which confirms some of the stereochemical assignments shown in table 1. Further details will be reported shortly.[9] We are currently extending this methodology further to related systems, and developing new synthetic procedures involving the use of homochiral acetals.



Entry	R^1	R^2	Solvent	% Yield[a]	d.e. or e.e. of **4** (configuration[b])	$[\alpha]_D^{20}$ (c, solvent)
1	Menthyl	$-CH_2CH_3$	$CHCl_3$	48	>95[c] (S)	-26.7 (1.0,EtOH)
2	Menthyl	$-CH_2CN$	$CHCl_3$	47	35[c] (S)	-36.1 (1.0,EtOH)
3	Menthyl	$-CH_2CH(OCH_3)_2$	$CHCl_3$	42	<10[c]	-48.3 (1.0,EtOH)
4	CH_3	$-CH_2CH(OCH_3)_2$	$CHCl_3$	49	40[d] (S)	+13.2 (1.0,EtOH)
5	CH_3	$-CH_2CH(OCH_3)_2$	^tBuOMe	45	>95[d] (R)	-53.5 (3.1,MeOH)
6	CH_3	$-CH_2CH(OEt)_2$	$CHCl_3$	46	30[d] (R)	-15.6 (1.0,EtOH)
7	CH_3	$-CH_2CH(OEt)_2$	^tBuOMe	49	>95[d] (R)	-31.9 (1.0,EtOH)
8	CH_3	$-CH_2CH(OBn)_2$	$CHCl_3$	No reaction	-----	-----
9	CH_3	$-CH_2CH(OBn)_2$	^tBuOMe	48	65[d] (R)	-20.2 (1.0,EtOH)
10	CH_3	$-(CH_2)_2CH(OMe)_2$	^tBuOMe	45	>95 (R)	-9.9 (1.3, EtOH)
11	CH_3	$-^nBu$	$CHCl_3$	No reaction	-----	-----
12	CH_3	$-^nBu$	^tBuOMe	47	>95[d] (R)	-41.5 (1.6, $CHCl_3$)
13	CH_3	$-(CH_2)_2OSiEt_3$	$CHCl_3$	42	20[d] (R)	n.d.
14	CH_3	$-(CH_2)_2OSiEt_3$	^tBuOMe	47	81[d] (R)	-48.7 (1.7, $CHCl_3$)
15	CH_3	$-(CH_2)_3OSiEt_3$	$CHCl_3$	46	25[d] (R)	n.d.
16	CH_3	$-(CH_2)_3OSiEt_3$	^tBuOMe	48	85[d] (R)	-36.2 (2.9, $CHCl_3$)
17	CH_3		$CHCl_3$	43	88[c] (R)	+20.1 (2.1, $CHCl_3$)

[a]Standard conditions: 100mg substrate, phosphate buffer (pH 7, 2ml), solvent (0.5ml), PFL (2mg), 30°C, 2h; reaction had generally proceeded to 50% (±5%) as determined by ^1H NMR; [b]tentatively assigned using literature model[13]; [c]Determined by ^1H NMR; [d]Determined by ^1H NMR using (+)-Eu(hfc)$_3$ or (-)-2,2,2-trifluoro-1-(9-anthryl)ethanol.

Table 1. Results of hydrolysis experiments.

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

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