Pergamon

Tetrahedron Letters, Vol. 36, No. 38, pp. 6961-6964, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4039/95 $9.50+0.00

0040-4039(95)01380-6

Enantioselective Enzymatic Synthesis of the Anti-Viral Agent Lamivudine (3TC™†).

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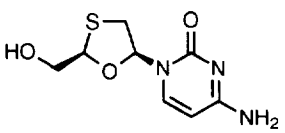
Abstract: A novel enzymatic resolution of α-acetoxysulfides has been used as the key step in the synthesis of the important antiviral nucleoside analogue lamivudine. The synthesis proceeds *via* a configurationally stable hemithioacetal which cyclises *in situ* to form the required oxathiolane nucleus, which can then be converted into the target nucleoside in 4 steps.

Our continued interest in reactive organosulfur intermediates[1] has led us to embark on a programme to develop an efficient enantioselective route to lamivudine (1), a highly promising drug candidate for HIV[2] and HBV[3] infections. Although the two enantiomers have similar potencies, the lower toxicity of the "unnatural" enantiomer means that enantioselective routes are of considerable importance, particularly if they are amenable to large scale synthesis. Previous syntheses of the (+)-isomer have used D-mannose[4] and D-galactose[5] as the source of chirality, whereas the desired (-)-isomer is available from L-gulose[6] and (+)-thiolactic acid.[7] A procedure using a lipase resolution of an oxathiolane intermediate has recently been reported[8] as has a large scale resolution of (±)-(1).[9] The 5-fluoro derivative of (1) has been resolved by lipase hydrolysis of the corresponding butyrate ester.[10]

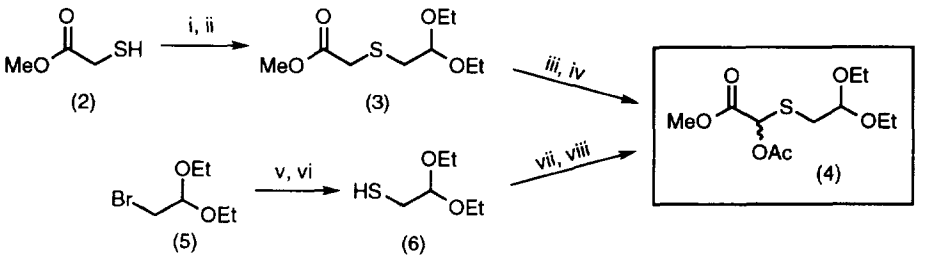


Lamivudine (1)

The enantioselective synthesis of lamivudine (1) provides a considerable challenge to the synthetic chemist due to the presence of two acetal chiral centres, both sharing the same oxygen atom. Current approaches for the synthesis of homochiral S,O-acetals[11] include the use of 7-thiomenthol originally reported by Eliel,[12] and the asymmetric Pummerer reaction.[13] Despite the particularly encouraging work recently reported by Kita *et al.*,[13a-c] our attempts at using an approach based on the asymmetric Pummerer reaction gave only minimal

chirality transfer from the sulfoxide, and was also limited due to problems accessing the required homochiral sulfoxide precursor.[14] We have recently developed an alternative strategy for the preparation of homochiral α-acetoxysulfides, based on a *pseudomonas fluorescens* lipase resolution.[15] We now describe how we have used this new methodology for the synthesis of lamivudine.

The key intermediate α-acetoxysulfide (4) could be prepared by two routes (scheme 1). Coupling of the sodium salt of methyl 2-mercaptoacetate (2) with bromoacetaldehyde diethylacetal in DMF, followed by oxidation of sulfide (3) to the sulfoxide and Pummerer rearrangement (Ac_2O, NaOAc, 90 °C) gave (4) in 40% overall yield. Use of higher temperatures for the Pummerer rearrangement (120 °C) in the absence of NaOAc led to formation of an enol ether by loss of ethanol. Alternatively, conversion of bromoacetaldehyde diethylacetal into the xanthate ester, and treatment with ethylenediamine[16] gave the thiol (6). Addition of (6) to methyl glyoxalate[17] in CH_2Cl_2 in the presence of 4Å molecular sieves gave an isolable hemithioacetal ((±)-(9)) which was usually acetylated *in situ* using Ac_2O and pyridine in CH_2Cl_2 with catalytic 4-(N,N-dimethylamino)pyridine (DMAP). This route gave a better overall yield (63%), however problems with oxidation of the thiol (6) to the disulfide led to variable yields in some cases. The dimethyl and dibenzylacetals (7) and (8) were prepared by analogous routes.



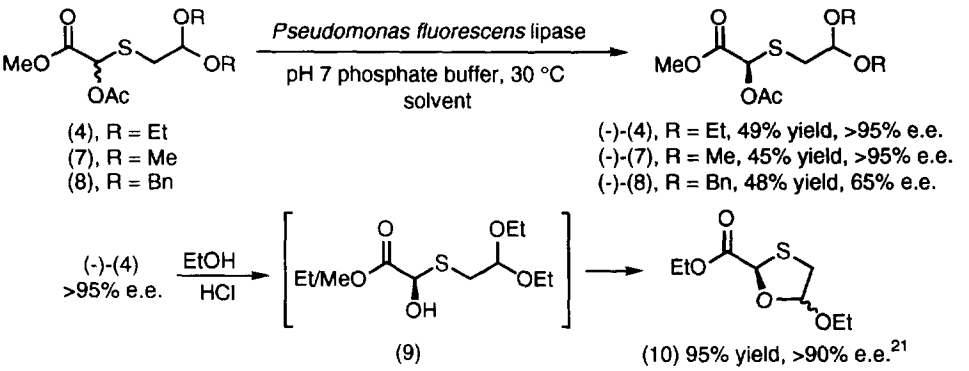
Reagents and conditions: i) Na, DMF; ii) $BrCH_2CH(OEt)_2$, 52%; iii) *m*-CPBA, CH_2Cl_2, 96%; iv) Ac_2O, NaOAc, 90 °C, 81%; v) KSC(S)OEt, acetone, 95%; vi) $H_2NCH_2CH_2NH_2$, 91%; vii) MeOC(O)CHO, CH_2Cl_2, 4Å sieves; viii) Ac_2O, pyridine, DMAP, 73%.

Scheme 1.

The α-acetoxysulfide (4) can be considered as being similar to the acetate derivative of a secondary alcohol, and as such is a potential candidate for enzymatic resolution using a lipase catalyst.[18] Our initial attempts at resolution of (4) using *Pseudomonas fluorescens* lipase[15] (pH 7 phosphate buffer, 30 °C, 2h.) gave disappointing enantiomeric excesses (e.e.s) with $CHCl_3$ cosolvent, however switching to ^{t}BuOMe resulted in a dramatic increase in e.e. (scheme 2). Very similar effects were observed in the case of the dimethylacetal (7). In the case of the dibenzylacetal (8) no reaction was observed in $CHCl_3$, however again switching to ^{t}BuOMe restored reactivity but with lower enantiomeric excess compared to the methyl and ethyl acetals. These, we believe, are the first examples of the use of an enzyme for the direct resolution of S,O-acetal chiral centres, and we are currently investigating this reaction in more detail, including other acetal systems.[18] The stereochemical assignments of the products are in accord with the literature model,[19] and have been confirmed by the synthesis of lamivudine (*vide infra*).

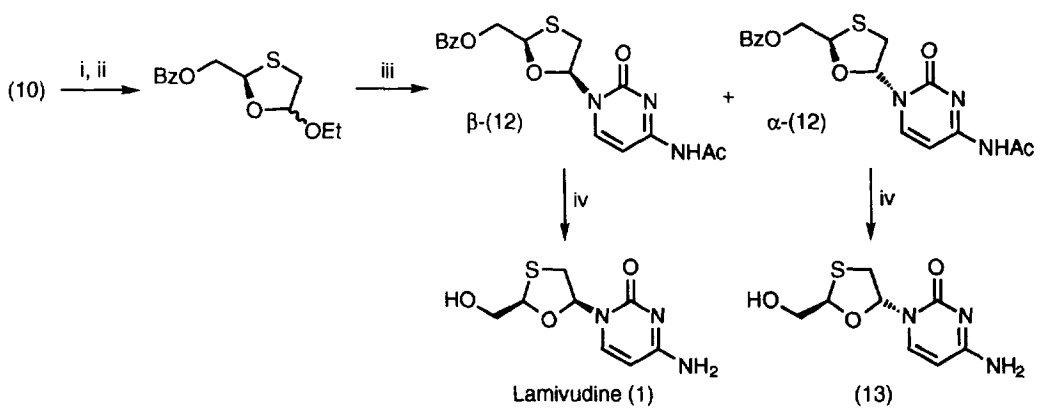
For our synthetic route to be viable, we needed to be able to convert (-)-(4) into the "sugar" ring with retention of stereochemistry at the resolved chiral centre. Although relatively little is known about the stereochemical stability of hemithioacetals (e.g. 9),[20] we believed that under acidic conditions any racemisation processes (either *via* dissociation or enolisation) should be sufficiently slow to allow cyclisation onto an adjacent acetal functionality. Thus treatment of (4) with HCl in dry ethanol induces acetate removal by transesterification to give the hemithioacetal (9) which cyclises *in situ* to the oxathiolane (10) with only a minor deterioration in the

high level of stereochemical integrity obtained from the enzymatic reaction (scheme 2). Concomitant transesterification of the methyl ester by EtOH solvent is also observed. This reaction can also be carried out in dry MeOH (66% yield), however the ethanol system gave significantly higher yields and selectivities.



Scheme 2.

Completion of the synthesis of lamivudine follows conventional nucleoside protocols (scheme 3). Reduction of the ethyl ester (10) and protection as the benzoate proceeded in 84% overall yield. Introduction of the base (TMSOTf, silylated N-acetyl cytosine (11), MeCN) gave the acetylated cytidine derivative (12) as a 1:1 mixture of α and β anomers, which could be separated by chromatography (SiO_2, 2:1 EtOAc/$CHCl_3$ to 100% EtOAc eluent). The individual anomers were then deprotected (NH_3/MeOH) to give (1) (87.9% e.e.[21]), and its α-anomer (13) (94.2% e.e.[21]) both in excellent yield. The optical rotations and chiral HPLC analyses of the products are consistent with previous literature reports and with those of authentic samples, and provide further verification of the literature model for the enzymatic resolution.[19,22]



Reagents and conditions: i) $LiBH_4$, ^iPrOH (cat.), THF, 96%; ii) BzCl, pyridine, CH_2Cl_2, 88%; iii) Silylated N-acetyl cytosine (11), TMSOTf, CH_3CN, 43%; iv) NH_3, MeOH, 98% (both anomers).

Scheme 3.

In conclusion, we have utilised our recently developed methodology for the enzymatic resolution of α-acetoxysulfides in an enantioselective synthesis of the important antiviral agent lamivudine. We are currently extending this methodology further to related systems, and developing new synthetic procedures involving the use of homochiral acetals.

Acknowledgements: we thank Glaxo Research and Development for a postdoctoral fellowship (JM),

and Glaxo Research and Development and the EPSRC for a CASE award (SB). We also thank Jon Cook and Nigel Metcalfe (GRD) for the chiral HPLC analyses.

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

References.

1.Westwell, A.D.; Thornton-Pett, M.; Rayner, C.M. *J. Chem. Soc., Perkin Transactions 1,* **1995**, 847; Pickersgill, I.F.; Marchington, A.P.; Thornton-Pett, M.; Rayner, C.M. *J. Chem. Soc., Chem. Commun.*, **1995**, 647; Archer, N.J.; Rayner, C.M.; Bell, D.; Miller, D. *Synlett*, **1994**, 617; Gill, D.M.; Pegg, N.A.; Rayner, C.M. *J. Chem. Soc., Perkin Transactions 1,* **1993**, 1371.
2.Coates, J.A.V.; Cammack, N.; Jenkinson, H.J.; Mutton, I.M.; Pearson, B.A.; Storer, R.; Cameron, J.; Penn, C.R. *Antimicrob. Agents and Chemotherap.*, **1992**, *36*, 202; Schinazi, R.F.; Chu, C.K.; Peck, A.; McMillan, A.; Mathis, R.; Cannon, D.; Jeong, L.-S.; Beach, J.W.; Choi, W.-B.; Yeola, S.; Liotta, D.C. *Antimicrob. Agents and Chemotherap.*, **1992**, 672.
3.Doong, S.-L.; Tsai, C.-H.; Schinazi, R.F.; Liotta, D.C.; Cheng, Y.-C. *Proc. Natl. Acad. Sci. USA*, **1992**, *88*, 8495.
4.Chu, C.K.; Beach, J.W.; Jeong, L.S.; Choi, B.G.; Comer, F.I.; Alves, A.J.; Schinazi, R.F. *J. Org. Chem.*, **1991**, *56*, 6503.
5.Jeong, L.S.; Alves, A.J.; Carrigan, S.W.; Kim, H.O.; Beach, J.W.; Chu, C.K. *Tetrahedron Lett.*, **1992**, *33*, 595.
6.Beach, J.W.; Jeong, L.S.; Alves, A.J.; Pohl, D.; Kim, H.O.; Chang, C.-N.; Doong, S.-L.; Schinazi, R.F.; Cheng, Y.-C.; Chu, C.K. *J. Org. Chem.*, **1992**, *57*, 2217.
7.Humber, D.C.; Jones, M.F.; Payne, J.J.; Ramsey, M.V.J.; Zacharie, B.; Jin, H.; Siddiqui, A.; Evans, C.A.; Tse, H.L.A.; Mansour, T.S. *Tetrahedron Lett.*, **1992**, *33*, 4625.
8.Cousins, R.P.C.; Mahmoudian, M.; Youds, P.M. *Tetrahedron Asymm.*, **1995**, *6*, 393.
9.Mahmoudian, M.; Baines, B.S.; Drake, C.S.; Hale, R.S.; Jones, P.; Piercey, J.E.; Montgomery, D.S.; Purvis, I.J.; Storer, R.; Dawson, M.J.; Lawrence, G.C. *Enzyme Microb. Technol.*, **1993**, *15*, 749.
10.Hoong, L.K.; Strange, L.E.; Liotta, D.C.; Koszalka, G.W.; Burns, C.L.; Schinazi, R.F. *J. Org. Chem.*, **1992**, *57*, 5563.
11.De Voss, J.J.; Sui, Z. *Tetrahedron Lett.*, **1994**, *35*, 49; Kim, S.; Park, J.H.; Lee, J.M. *Tetrahedron Lett.*, **1993**, *34*, 5769; Kusche, A.; Hofmann, R.; Munster, I.; Keiner, P.; Bruckner, R. *Tetrahedron Lett.*, **1991**, *32*, 467; Masaki, Y.; Serizawa, Y.; Kaki, K. *Chemistry Lett.*, **1985**, 1933; Sato, T.; Kobayashi, T.; Gojo, T.; Yoshida, E.; Otera, J.; Nozaki, H.; *Chemistry Lett.*, **1987**, 1661; Guindon,Y.; Bernstein, M.A.; Anderson, P.C.; *Tetrahedron Lett.*, **1987**, *28*, 2225; Kim, S.; Park, J.H.; Lee, S. *Tetrahedron Lett.*, **1989**, *30*, 6697; Otera, J. *Synthesis*, **1988**, 95; Cohen, T.; Bhupathy, M. *Acc. Chem. Res.*, **1989**, *22*, 152.
12.Eliel, E.L.; Frye, S.V.; Hortelano, E.R.; Chen, X.; Bai, X. *Pure Appl. Chem.*, **1991**, *63*, 1591 and refs. cited therein; Nishida, M.; Nakaoka, K.; Ono, S.; Yonemitsu, O.; Nishida, A.; Kawahara, N.; Takayanagi, H. *J. Org. Chem.*, **1993**, *58*, 5870; Solladie, G; Lohse, O. *Tetrahedron: Asymm.*, **1993**, *4*, 1547; Wei, J.; Hutchins, R.O.; Prol Jr., J. *J. Org. Chem.*, **1993**, *58*, 2920.
13.a) Kita, Y.; Shibata, N.; Kawano, N.; Kukui, S.; and Fujimori, C. *Tetrahedron Lett.*, **1994**, *35*, 3575; b) Kita, Y.; Shibata, N.; Yoshida, N.; Fukui, S.; Fujimori, C. *Tetrahedron Lett.*, **1994**, *35*, 2569; c) Kita, Y.; Shibata, N.; Yoshida, N. *Tetrahedron Lett.*, **1993**, *34*, 4063. For an excellent comprehensive review on the Pummerer reaction see: De Lucchi, O.; Miotti, U.; Modena, G. *Organic Reactions*, **1990**, *40*, 157.
14.Milton, J.; Rayner, C.M.; Jones, M.F. unpublished results. A related racemic synthesis using a Pummerer rearrangement as a key step has been carried out, see: Dwyer, O, *submitted for publication.*
15.Milton, J.; Brand, S.; Jones, M.F.; Rayner, C.M. *submitted for publication.*
16.Djerassi, C.; Gorman, M.; Markley, F.X.; Oldenberg, E.B. *J. Amer. Chem. Soc.*, **1955**, *77*, 568; Mori, K.; Nakamura, Y.; *J. Org. Chem.*, **1969**, *34*, 4170.
17.Kelly, T.R.; Schmidt, T.E.; Haggerty, J.G. *Synthesis*, **1972**, 544.
18.a) Roberts, S.M.; Wiggins, K.; Casy, G.; Phythian, S. *Preparative Biotransformations,* J. Wiley, Chichester, 1992; b) Davies, H.G.; Green, R.H.; Kelly, D.R.; Roberts, S.M. *Biotransformations in Preparative Organic Chemistry*, Best Synthetic Methods Series, Academic Press: London, 1992. There is a single report of the enzymatic hydrolysis of chloral acetyl methyl acetal: Chenevert, R.; Desjardins, M.; Gagnon, R. *Chemistry Lett.*, 1990, 33; α-Hydroxyphosphonates have also been resolved using a related procedure: Drescher, M.; Li, Y.-F.; Hammerschmidt, F. *Tetrahedron*, **1995**, *51*, 4933.
19.Cygler, M.; Grochulski, P.; Kazlauskas, R.J.; Schrag, J.D.; Bouthillier, F.; Rubin, B.; Serreqi, A.N.; Gupta, A.J. *J. Amer. Chem. Soc.*, **1994**, *116*, 3180 and refs. cited therein.
20.Linderman, R.J.; Tennyson, S.D.; Shultz, D.A. *Tetrahedron Lett.*, **1994**, *35*, 6437.
21.Enantiomeric excess determinations were carried out by ^{1}H NMR using Eu(hfc)$_3$ or (-)-2,2,2-trifluoro-1-(9-anthryl)ethanol, except for the final products (-)-(1) and (+)-(13) which were determined by chiral HPLC.
22.All new compounds were characterised by ^{1}H and ^{13}C NMR, IR, and mass spectra, and gave satisfactory elemental analysis and/or accurate mass spectra unless otherwise stated.

(*Received in UK* 3 *July* 1995; *accepted* 21 *July* 1995)