indicate that this electroreductive method is effective for stereoselective synthesis of a variety of phenylcyclopropanols 8.

It has been reported that the treatment of 8a with acid or base formed cyclopropane ring-opened products.[32,33] It was found in the present study that 8a yielded similar ring-opened products with much better selectivity under modified reaction conditions.

The base-catalyzed ring-opening reaction of phenylcyclopropanols was successfully applied to the synthesis of *ar*-dihydroturmerone (R = CH_2CHMe_2) (11) and curcumone (R = Me) (12).[34] Specifically, the cathodic cyclocoupling of *p*-methyl-α-methylstyrene with methyl isovalerate and methyl acetate gave phenylcyclopropanol derivatives 9 and 10, respectively. Addition of *t*-BuOK (0.1 equiv based on 9 or 10) to a solution of 9 and 10 in *t*-BuOH gave 11 and 12, respectively (Scheme V).

Supplementary Material Available: Experimental procedures for new compounds (4 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(32) DePuy, C. H.; Breitbeil, F. W. *J. Am. Chem. Soc.* **1963**, *85*, 2176.
(33) Gibson, D. H.; DePuy, C. H. *Chem. Rev.* **1974**, *6*, 605.
(34) The structures of 11[35] and 12[36] were determined by comparison of their spectrscopic values with those of authentic samples.
(35) Kashima, C.; Yamamoto, Y. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1735.
(36) Ho, T. L. *Synth. Commun.* **1981**, *11*, 579.

Enzyme-Mediated Enantioselective Preparation of Pure Enantiomers of the Antiviral Agent 2′,3′-Dideoxy-5-fluoro-3′-thiacytidine (FTC) and Related Compounds

Lee K. Hoong, Luise E. Strange, and Dennis C. Liotta*

Department of Chemistry, Emory University, Atlanta, Georgia 30322

George W. Koszalka* and Charlene L. Burns

Division of Experimental Therapy, Burroughs Wellcome Co., 3030 Cornwallis Road, Research Triangle Park, North Carolina 27709

Raymond F. Schinazi

Department of Pediatrics, Emory University School of Medicine and Veterans Administration Medical Center, 1670 Clairmont Road, Decatur, Georgia 30033

Received July 8, 1992

Summary: Racemic 2′,3′-dideoxy-3′-thianucleosides were resolved by enzyme-catalyzed hydrolysis of their butyrate ester derivatives.

Recently, we required the enantiomers of the important antiviral agents BCH-189 (2′,3′-dideoxy-3′-thiacytidine, 1) and FTC (2′,3′-dideoxy-5-fluoro-3′-thiacytidine, 2) for biological evaluation.[1] Initial attempts to resolve the enantiomers by either classical techniques or enantioselective synthesis were unsuccessful.[2] Since enzymes have been exploited for biocatalytic resolutions, we examined their potential to resolve the racemates of 1 and 2.[3] Lipases, esterases, and proteases were chosen because of their commercial availability, relatively low cost, and tolerance for a wide class of substrates. Since nucleosides have been typically synthesized from chiral, nonracemic precursors (e.g., carbohydrates or other naturally-occurring nucleosides), little information exists regarding the enantioselective enzyme-catalyzed hydrolysis of racemic nucleosides.[4] The results of our study are presented herein.

Enantioselective Enzyme-Catalyzed Hydrolysis of FTC Esters. *O*-Acyl derivatives of sulfur-containing nucleosides (3b–e and 4a–e) were prepared either by O-acylation of the 5′-hydroxyl group or by tin-mediated coupling of the corresponding acetate precursor 7a–e with the appropriate cytosine base (5 or 6; Scheme I).[5] A

(1) (a) Choi, W.-B.; Wilson, L. J.; Yeola, S.; Liotta, D. C.; Schinazi, R. F. *J. Amer. Chem. Soc.*, **1991**, *113*, 9377. (b) 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. (c) Doong, S.-L.; Tsai, C.-H.; Schinazi, R. F.; Liotta, D. C.; Cheng, Y.-C. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 8495. (d) Doong, S.-L.; Tsai, C.-H.; Schinazi, R. F.; Liotta, D.; Cheng, Y. C. *Interscience Conference on Antimicrobial Agents and Chemotherapy*; Chicago, IL, Sept 29–Oct 2, 1991. (e) Schinazi, R. F.; Liotta, D. C.; Choi, W.-B.; Peck, A.; McClure, H. M.; Boudinot, F. D.; Sommadosi, J.-P.; Davis, M.; Furman, P. A.; Painter, G. *National Collaborative Drug Discovery Group, Frontiers in HIV Therapy*; San Diego, CA, Nov 3–7, 1991. (f) 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 Chemother.* **1992**, *36*, 672.

(2) Formation of chiral salts between various nucleoside derivatives and camphorsulfonic acid or tartaric acid derivatives was examined, but no detectable enrichment was observed in repeated attempts at crystallization. Efforts directed at enantioselective synthesis were thwarted by racemization during a crucial step involving the formation of the nucleoside via a tin-mediated coupling between the acetate 7 and the pyrimidine base.

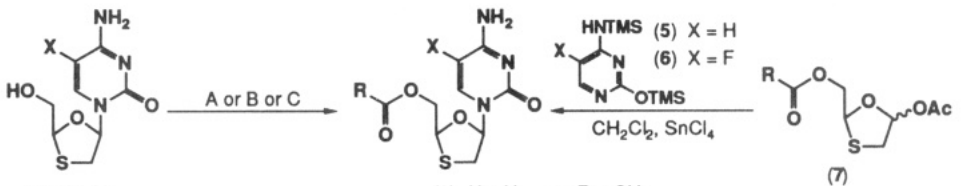
(3) For reviews of applications of enzymes in organic synthesis, see: (a) Santaniello, E.; Ferraboschi, P.; Grisenti, P.; Manzocchi, A. *Chem. Rev.* **1992**, *92*, 1071. (b) Klibanov, A. M. *Acc. Chem. Res.* **1990**, *23*, 114. (c) Wong, C.-H. *Chemtracts* **1990**, *3*, 91. (d) Zhu, L.; Tedford, M. C. *Tetrahedron* **1990**, *46*, 6587. (e) Whitesides, G. M.; Wong, C. *Aldrichim. Acta* **1983**, *16*, 27. (f) Turner, N. J. *Nat. Prod. Rep.* **1989**, *6*, 625. (g) Sih, C. J.; Wu, S. In *Topics in Stereochemistry*; Eliel, E. L., Wilen, S. H., Eds.; John Wiley and Sons: New York, 1989; Vol. 19, p 63. (h) Walpole, C. S. J.; Wrigglesworth, R. *Nat. Prod. Rep.* **1989**, 311. (i) Butt, S.; Roberts, S. M. *Chem. Britain* **1987**, 127. (j) Jones, J. B. *Tetrahedron* **1986**, *42*, 3351. (k) Mulzer, J. In *Organic Synthesis Highlights*; Mulzer, J., Altenbach, H., Braun, M., Krohn, K., Reissig, H., Eds.; VCH: New York, 1991; p 216. (l) Mulzer, J. In *Organic Synthesis Highlights*; Mulzer, J., Altenbach, H., Braun, M., Krohn, K., Reissig, H., Eds.; VCH: New York, 1991; p 207.

(4) Of the studies reported, almost all have focused on *regioselective* hydrolyses for differentiating two or more available ester functional groups. For a recent attempt at enantioselective lipase-mediated hydrolysis of carbohydrate-like substrates which can be utilized for the synthesis of C-nucleosides, see: Hultin, P. G.; Mueseler, F.-J.; Jones, J. B. *J. Org. Chem.* **1991**, *56*, 5375. For an example of enantioselective resolution by enzymatic deamination of purine nucleosides, see: Secrist, J. A., III; Montgomery, J. A.; Shealy, Y. F.; O'Dell, C. A.; Clayton, S. J. *J. Med. Chem.* **1987**, *30*, 746.

(5) For the stereoselective preparation of the β-anomer of BCH-189 1, see ref 1a. A detailed process for the preparation of FTC 2 and its 5′-*O*-(acyloxy) derivatives on a multigram scale will be reported elsewhere.

0022-3263/92/1957-5563$03.00/0

Scheme I



(1) X = H
(2) X = F

(3) X = H
(4) X = F

a: R = CH_3
b: R = CH_3CH_2
c: R = $CH_3(CH_2)_2$
d: R = $CH_3(CH_2)_3$
e: R = $(CH_3)_3C$

A: (i) HCl, 23 °C; (ii) AcOH, AcCl, 23 °C
B: (i) $(RCO)_2O$, Δ; (ii) HCl, MeOH, 23 °C
C: (i) RC(O)Cl, pyridine, 23 °C; (ii) HCl, MeOH, 23 °C

Table I. Enantioselectivity of Various Enzymes in the Hydrolysis of FTC Butyrate 4c

entry	enzyme	% convsn	% ee[7] (ester remaining)	enantiomeric preference[8]
1	*Aspergillus sojae* protease	54	55	(+)
2	*Bacillus subtilis* protease	61	10	(+)
3	*Candida cylindracea* lipase	52	7	(−)
4	Lipase AK	56	23	(−)
5	Lipase APF-12	69	16	(−)
6	Lipase AY-30	48	14	(−)
7	Lipase CE	65	50	(+)
8	Lipase PS-30	56	83	(−)
9	PLE	59	93	(+)
10	Pronase E	61	46	(+)
11	Protease M	63	60	(+)
12	*Pseudomonas* sp. lipase	66	33	(−)
13	Subtilisin	75	14	(+)
14	Triacylglycerol lipase	62	40	(+)

Table II. Effect of Varying Ester Acyl Group in the Enzyme-Catalyzed Hydrolysis of 3′-Thiacytidine Nucleosides

entry	enzyme	ester	R	% convsn	$t_{1/2}$ (h)[15]	% ee[7] (ester remaining)
1	PLE	**3b**	*n*-C_2H_5	50	71	>99
2		**3c**	*n*-C_3H_7	54	17	>99
3		**3e**	*t*-C_4H_9		≫400	ND[16]
4		**4a**	CH_3	61	50	70
5		**4b**	*n*-C_2H_5	56	2	93
6		**4c**	*n*-C_3H_7	52	2	98
7		**4c**	*n*-C_3H_7	58	2	>99
8		**4d**	*n*-C_4H_9	60	3	>99
9		**4e**	*t*-C_4H_9		≫400	ND[16]
10	PS-800	**4a**	CH_3	55	328	86
11		**4c**	*n*-C_3H_7	58	50	94

number of enzymes were initially evaluated for both reactivity and enantioselectivity using the 5′-*O*-butyrate derivative **4c** as the substrate (Table I).[6] The time-course for ester hydrolysis was monitored by HPLC. The enantioselectivity was determined by chiral HPLC analysis.[7] The sense of the enantioselectivity[8] was dependent on the enzyme (cf. entry 8 vs entry 9; Table I). Although a range of enantioselectivity was observed, two enzymes had resolutions ≥80% ee (entries 8 and 9; Table I). On the basis of these results, as well as availability considerations, we chose to examine further the hydrolysis reactions of pig liver esterase (PLE, Amano International Enzyme Co., Inc. (Troy, VA)) as well as those of PS-800 (Amano), a purified form of PS-30 (entry 9; Table I).[9] Subsequent studies, under slightly modified conditions (vide infra), revealed that these biocatalysts promote a highly enantioselective hydrolysis of the nucleoside esters (Table II).

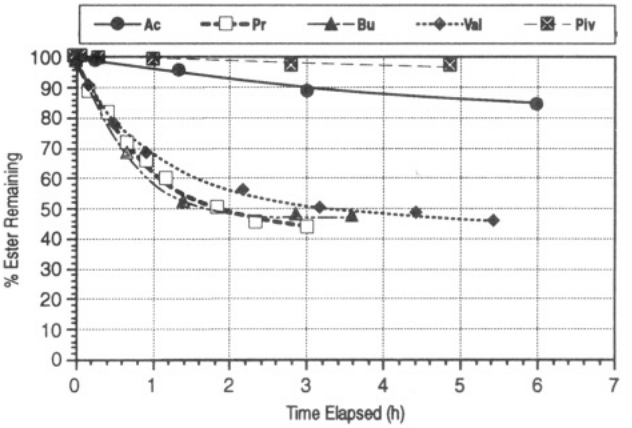


Figure 1. PLE-catalyzed hydrolyses of various FTC ester acyl groups.

Variations in the ester acyl group influenced the rate of enzymatic hydrolysis (Figure 1).[10] In the case of PLE, the butyrate ester **4c** hydrolyzed much faster than the 5′-*O*-acetate **4a**, but at a comparable rate to the 5′-*O*-valerate and -propionate esters. Esters with α-branched acyl groups, such as **4e**, were very poor substrates, exhibiting extremely low rates of hydrolysis. A similar trend was observed for PS-800. In addition, the ester derivatives of BCH-189 were hydrolyzed at a significantly lower rate than the corresponding FTC derivatives (cf. entry 1 vs entry 5; Table II). Although there are small variations in the enantioselectivity observed with changes in the acyl group, the enantioselectivity observed in most cases was excellent (entries 1, 2, 5–8, 11; Table II).

The hydrolysis reactions were typically incubated at room temperature in 4:1 pH 8 buffer (0.05 M potassium phosphate)–CH_3CN with 0.03 M substrate and either 50

(6) These preliminary experiments were performed at pH 7 and a substrate concentration of 0.02 M in 25 mM $K_2H_2PO_4$ buffer containing 10% acetonitrile. Incubation was done at 40 °C. Aliquots were quenched in absolute ethanol and eluted directly on a Daicel Chiralpak AS column (0.46 × 25 cm) using 50:50 absolute EtOH/2-propanol at a flow rate of 0.8 mL/min. Under these conditions, (−)-**2**, (+)-**2**, (−)-**4c**, and (+)-**4c** eluted at 5.3, 7.1, 8.3, and 10.1 min, respectively.

(7) The enantiomers of FTC esters **4a–e** are resolved on a Daicel Chiralpak-AS HPLC column (0.46 × 25 cm) using 100% 2-propanol as eluant at a flow rate of 0.8 mL/min. The enantiomers were identified by a UV/vis detector at 265 nm or 280 nm. Under these conditions, (−)-**4c** eluted at 11.9 min while its (+)-enantiomer eluted at 16.9 min.

(8) The (+) and (−) convention utilized here refers to the sign of rotation of the *free nucleoside* that will result from the removal of the 5′-*O*-acyl group in the ester.

(9) PLE and PS-800 are commercially-available from Amano and are used without further purification.

(10) For additional accounts on the dependence of the ester alkyl group on lipase-mediated hydrolyses, see: (a) Fink, A. L.; Hay, G. W. *Can. J. Biochem.* **1969**, *47*, 353. (b) Jensen, R. G.; DeJong, F. A.; Clark, R. M. *Lipids* **1983**, *18*, 239. (c) Krisch, K. In *The Enzymes*; Boyer, P. D., Ed.; Academic Press: New York, 1971; Vol. 5, p 43. (d) Malhotra, O. P.; Philip, G. *Biochem. Z.* **1966**, *346*, 386. (e) Dixon, M.; Webb, E. C.; Thorne, C. J. R.; Tipton, K. F. *Enzymes*, Academic Press: New York, 1979; p 252 and references therein.

units of PLE or 2200 units of PS-800 per 0.1 g of ester.[11] CH_3CN was included to facilitate the solubility of the substrate in the buffer. If the reactions were performed under nonhomogeneous conditions, the enantioselectivities were much lower (ca. 40% ee) and difficult to reproduce. These results suggest that under nonhomogeneous conditions the rate of dissolution was rate determining such that the substrate/enzyme ratio was much smaller, thereby negating the enzymes' inherent kinetic preference for one of the enantiomers. Indeed, in the case of the pivalate and valerate esters, the low solubility of these esters required either additional amounts of CH_3CN and/or further dilution of the reaction mixture to effect complete solubility.[12] This problem was compounded by a significant reduction of enzyme activity with greater than 25% (v/v) CH_3CN. Hydrolysis at elevated temperatures (35–45 °C) resulted in a reduction in the percent conversion, which was presumably due to the decreased stability of the enzyme under these conditions.

The recovery of unreacted ester was simple and effective. Thus, the hydrolysis reactions were quenched with $CHCl_3$; the aqueous and organic phases were separated, and the aqueous layer was extracted with $CHCl_3$. When the substrates in question were relatively lipophilic (i.e., the 5′-*O*-butyrate, valerate, or pivalate), these extractions efficiently recover the unreacted ester with only traces (≤2%) of the free nucleoside present. The efficiency of the recovery was ≥75%. On the basis of reactivity, solubility, and workup consideration, the 5′-*O*-butyrate ester was optimal with PLE for achieving an efficient biocatalytic resolution.

We evaluated the anti-HIV activity and toxicity of the enantiomers of FTC and BCH-189. In each case the (−)-enantiomers proved to be both more active and less toxic than their (+)-counterparts.[13] This activity/toxicity profile is particularly striking for (−)-FTC which exhibits potent anti-HIV activity but no marked toxicity in a large array of cellular assays.[14]

X-ray crystallographic analysis of (−)-FTC determined the structure to have the "unnatural" L-absolute configuration.[17] Metabolism studies have demonstrated that (−)-FTC is efficiently converted in vivo to its corresponding triphosphate.[1e] These two observations suggest an intriguing rationale for the low toxicity of (−)-FTC. Although it was readily anabolized to its active form by cellular kinases, (−)-FTC is probably not recognized as an effective substrate by other cellular enzymes because of its "unnatural" absolute configuration. Thus, it does not interfere with normal cellular processes and, hence, does not exhibit toxicity. *If this hypothesis proves general, it would be an important new strategy for designing antiviral agents with low toxicity.*

In summary, a procedure has been developed for the highly enantioselective enzyme-catalyzed resolution of FTC and related sulfur-containing nucleosides, based on PLE-mediated hydrolysis of their butyrate ester derivatives. The use of butyrate esters facilitated the separation of the optically-enriched, unreacted substrate from the medium by an extraction with $CHCl_3$. PS-800 had complementary enantioselectivity with this class of substrates. The rates of ester hydrolysis by PS-800 were slower than those for PLE. This process was scaled to the preparation of multigram quantities of enantiomerically pure (−)-FTC, **2**. In view of the finding that the unnatural (−)-enantiomer of FTC exhibited an excellent activity/toxicity profile against HIV-1, methods providing ready access to these antipodes, such as the one we have described here, become increasingly important. Studies are currently underway to probe the scope of this methodology in terms of further variations in reaction conditions and substrate structure, including other natural and unnatural nucleosides.

Acknowledgment. Financial support was provided by grants AI-28731 and AI-31827 from the National Institutes of Health and the Department of Veterans Affairs. D.C.L. wishes to thank Dr. Paul Reider (Merck) for helpful advice regarding enzymatic hydrolyses. We are grateful to Dr. Jose Soria for conducting the large-scale enzymatic resolution.

Supplementary Material Available: A representative procedure for the enantioselective, enzyme-catalyzed hydrolysis of the 3′-thiacytidine nucleoside esters **3b**, **3c**, **3e**, and **4a–e** with the corresponding spectroscopic data for these compounds as well as FTC **2** (7 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(11) One unit of enzyme hydrolyzed 1 μmol of *o*-nitrophenylbutyrate (*o*-NPB)/min at pH 8.0, 25 °C. Assay mixtures contained: 1 mM *o*-NPB, 2% DMSO, 50 mM Tris-HCl, pH 8 and enzyme. Enzyme activity was determined at 420 nm ($\Delta\epsilon$ = 5000 M^{-1} cm^{-1}). A 50 mM stock solution of *o*-NPB was made fresh each day by diluting 17.5 μL of substrate up to 2 mL of DMSO.

(12) (a) The hydrolysis reaction with the valerate **4d** was conducted in 3:1 pH 8 buffer–CH_3CN. (b) The pivalate substrate **4e** was performed at 0.01 M (a 3-fold dilution). The resulting drastic reduction in the rate of hydrolysis was not, however, solely due to a dilution effect since hydrolysis of the butyrate ester **4c** under identical conditions proceeded at a much greater rate.

(13) The median effective concentration (EC_{50}) for (+)- and (−)-BCH-189 and (+)- and (−)-FTC was 0.2, 0.002, 0.84, and 0.008 μM in HIV-1 (strain LAV) in acutely infected human peripheral blood mononuclear (PBM) cells. Similar magnitude effects were observed for these compounds in CEM and MT-4 cells. None of the compounds were toxic to PBM cells when evaluated in concentrations up to 100 μM. However, in CEM cells, (+)-BCH-189 was toxic (IC_{50} = 2.7 μM).

(14) Other relevant cell lines which were assayed include MT-2, Vero, and human bone marrow cells.

(15) The $t_{1/2}$ is defined as the time taken for the hydrolysis reaction to reach 50% conversion.

(16) ND: not determined. The excessively lengthy reaction times prohibited the determination of the enantioselectivity of the hydrolysis reaction at >50% conversion.

(17) Van Roey, P.; Pangborn, W. A.; Schinazi, R. F.; Painter, G. R.; Liotta, D. C. *Antiviral Chem. Chemother.*, submitted.