Enzyme-Catalyzed Enantioselective Hydrolysis of Diacetylated (±)-1-(2′,3′-Dideoxy-3′-fluoroapio-β-furanosyl) Cytosine

Minsoo Song and Yonghyun Ahn*

Department of Chemistry, Dankook University, Seoul 140-714, Korea
Received September 1, 2000

Keywords : Nucleoside, Lipase, Chiral resolution.

As antiviral agents, the nucleoside derivatives fluorinated in sugar moiety are highly attractive compounds providing a source of new drugs.[1,2] For example, nucleocidine isolated from *Streptomyces calvus* has been shown to be a highly potent inhibitor of protein biosynthesis.[3] Nucloside analogues are among the most potent agent against HIV-1.[4] As their 5'-triphosphate metabolites, they are selective inhibitors of the viral reverse transcriptase.[5] The 2',3'-dideoxynucleoside class of antiviral agent has been investigated as a potent inhibitor of the human immunodeficiency virus (HIV).[6] In effort to synthesize novel fluorinated nucleoside derivative, the racemic mixture of (±)-1-(2',3'-dideoxy-3'-fluoroapio-β-furanosyl) cytosine [(±)-**1**] has been synthesized[7] and it could be used as an antiviral reagent. A more efficient activity test requires the optically pure isomer. To obtain the optically pure compound, the asymmetrical synthesis of these compounds involves a quite complicated process, and the overall yield of the reaction is usually very low. Recently, the various enzymes (lipases, esterases, and proteases) are commercially available that makes the enzymatic resolutions of racemic mixture become popular.[8]

We started our investigations by using the lipase to hydrolyze the diacetylated (±)-1-(2',3'-dideoxy-3'-fluoroapio-β-furanosyl) cytosine [(±)-**2**] in phosphate buffer. The substrate [(±)-**2**] does not dissolved in the buffer solution therefore a polar solvent should be used for the enzyme reaction. Based on the screening of organic solvents, *n*-butanol showed good solubility for (±)-**2** and it was the most suitable solvent for this enzyme reaction. The enantioselectivity of the enzyme reaction was determined on the basis of the rotation of the free nucleoside obtained from the removal of the acetyl group.[9] When acetone was used as a cosolvent, porcine liver lipase and porcine liver esterase showed good reactivity, but the optical purity of the product was not high. In the case of lipase AK, the reaction proceeded less stereoselective in acetone than in *n*-butanol. Among the tested enzymes, lipase AK (from Amano International Enzyme Co., Inc., Japan) showed the best enantioselectivity and reactivity.

In typical reaction condition, lipase AK (a lipase from *Pseudomonas fluorescens* sp.,) was added to a solution of (±)-**2** in 0.1 N phosphate buffer (pH = 7) and *n*-butanol at 25 °C, and the reaction mixture was stirred under aerobic conditions. The reaction was monitored with HPLC {Shimaz, C_{18}, 1 mL/min, 245 nm, MeOH : H_2O = 7 : 3 (v/v)} and then quenched by filtration of the enzyme through celite after which the product was freeze dried. The reaction mixture was separated by silica gel column chromatography. The compound **3** was treated with sodium methoxide in MeOH to eliminate the acetyl group, and the (+)-1-(2',3'-dideoxy-3'-fluoroapio-β-D-furanosyl) cytosine [(+)-**1**] was isolated in 43% yield with an optical purity of 93% ee. In a similar manner, the (–)-1-(2',3'-dideoxy-3'-fluoroapio-β-L-furanosyl) cytosine [(–)-**1**] was obtained in 40% yield with 92% ee.

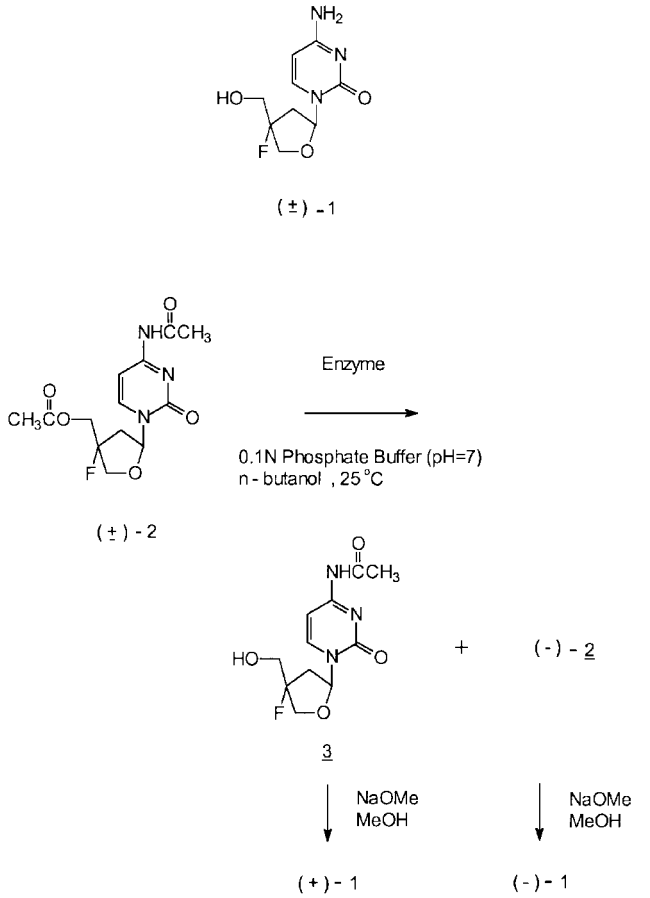
In conclusion, a procedure has been developed for the highly enantioselective enzyme-catalyzed resolution of (±)-1-(2',3'-dideoxy-3'-fluoroapio-β-furanosyl) cytosine based on lipase AK mediated hydrolysis of its acetyl ester derivative.

Experimental Section

The lipase AK, lipase-PS, and lipase AP-12 were purchased from Amano Enzyme Co., Inc., (Japan). The enzymes were used without further purification. ^{1}H NMR spectra were recored on a Bruker ARX-400 spectrometer (400 MHz) and are reported in ppm using residual undeuterated solvent as an internal standard. HPLC monitering of the enzyme mediated hydrolysis reactions were conducted using a ASTEC Cyclobond column (0.46 cm × 25 cm) using 0.2% triethylammonium acetate as eluant at 1 mL/min (observed at 254 nm). Optical rotations were measured using a Perkin Elmer 241MC polarimeter.

***N*-Acetylated (+)-1-(2',3'-dideoxy-3'-fluoroapio-β-furanosyl) cytosine (3)**. (±)-**2** (1 g, 3.1 mmol) was dissolved in 20 mL of pH 7 phosphate buffer (0.1 N)-*n*-butanol (3 : 1). The clear solution was treated with lipase AK (0.5 g, lipase from *Pseudomonas fluorescens* sp.). The progress of the reaction was monitored by HPLC. After 3h (50% conversion), the reaction mixture was quenched by filtration of the enzyme through celite and product was freeze dried. The reaction mixture was separated by silica gel column chromatography (CH_2Cl_2 : MeOH = 95 : 5). **3** was isolated in 44% yield (0.36 g) and (–)-2 was obtained in 42% yield (0.4 g).

3 ^{1}H NMR (DMSO -d_6) δ: 10.9 (s, 1H), 8.09 (d, 1H, *J* = 7.4 Hz), 7.18 (d, 1H, *J* = 7.2 Hz), 6.07 (t, 1H, *J* = 6.6 Hz), 5.28 (t, 1H, *J* = 5.4 Hz), 4.23 (dd, 1H, *J* = 35.2, *J′* = 10.4 Hz), 4.04 (dd, 1H, *J* = 21.2, *J′* = 11 Hz), 3.58-3.71 (m, 2H), 2.2-2.75 (m, 2H), 2.06 (s, 3H); ^{13}C NMR (DMSO-d_6) δ: 170.0, 161.5, 153.4, 144.6, 105.8, 102.2, 94.3, 87.4, 74.3,



Scheme 1

Table 1. Enzymatic Hydrolysis of compound (±)-**2**

Enzyme	Solvent	Time (hr)	(+)-1		(–)-1	
			Yield (%)	ee (%)	Yield (%)	ee (%)
lipase-AK	acetone	48	37	48	35	34
lipase-PS	*n*-butanol	3	34	77	30	68
Prozyme-6	*n*-butanol	1	35	27	19	45
lipase AP-12	*n*-butanol	1	18	36	27	41
lipase-AK	*n*-butanol	3	43	93	40	92

[a] The yield was based on the isolation of (+)-**1** and (–)-**1**.

73.8, 61.0, 60.5, 23.2

(+)-1-(2',3'-Dideoxy-3'-fluoroapio-β-D-furanosyl) cytosine [(+)-1]. The compound **3** (0.36 g, 1.3 mmol) was dissolved in 20 mL of MeOH and then added 0.15 g (2.7 mmol) of NaOMe. The resulting mixture was stirred at room temperature for 30 min. Solvent was removed by rotary evaporation to give a crude white solid. The crude compound was purified with silicagel column chromatography ($CHCl_3$: MeOH = 7 : 3). **(+)-1** was isolated as a white solid in 97% (0.27 g) yield. $[\alpha]^{20}_D$ + 45.1 [ref. $[\alpha]^{20}_D$ + 48.6 (0.17, MeOH)]. ^{1}H NMR (DMSO-d_6) δ: 7.61 (d, 1H, J = 7.4 Hz), 7.16-7.12 (m, 2H), 6.11 (t, 1H, J = 6.5 Hz), 5.70 (d, 1H, J = 7.4 Hz), 5.23 (t, 1H, J = 5.7 Hz), 4.19 (dd, 1H, J = 35, J' = 10.5 Hz), 3.94 (dd, 1H, J = 21, J' = 10.7 Hz), 3.71-3.63 (m, 2H), 2.48-2.20 (m, 2H); ^{13}C NMR (DMSO-d_6) δ: 166.6, 157.1, 141.5, 105.4, 103.6, 95.2, 88.6, 75.3, 75.0, 62.9, 62.7.

Acknowledgment. The authors wish to acknowledge the financial support of the Chong Kun Dang Pharmaceutical Co.

References

1. Graul, A.; Silvestre, J.; Castaner, J. *Drugs of the Future* **1998**, *23*, 1176.
2. (a) Jenkins, D.; Verheyden, J. P. H.; Moffatt, J. G. *J. Am. Chem. Soc.* **1976**, *98*, 3346. (b) Owen, G. R.; Verheyden, J. P. H.; Moffait, J. G. *J. Org. Chem.* **1976**, *41*, 3010. (c) Yang, C. O.; Kurz, W.; Eugui, E. M.; MeRoberts, M. J.; Verheyden, J. P. H.; Kurz, L. J.; Walker, K. A. M. *Tetrahedron Lett.* **1992**, *33*, 41. (d) Lipshutz, B. H.; Sharma, S.; Dimock, S. H.; Behling, J. R. *Synthesis* **1992**, 191.
3. (a) Florini, J. R.; Bird, H. H.; Bell, P. H. *J. Biol. Chem.* **1966**, *241*, 1091. (b) Thomass, S. O.; Singleton, V. L.; Lowery, J. A.; Sharpe, R. W.; Pruess, L. M.; Porter, J. N.; Mowat, J. H.; Bohonos, N. *Antibiot. Annu*. **1956**, 716.
4. (a) De Clercq, E.; *Tr. Pharm. Sci.* **1987**, *8*, 339. (b) Mitsuya, H.; Broder, S. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 1911.
5. Cheng, Y. C.; Dutschman, G. E.; Bastow, K. F.; Sarngadharan, M. G.; Ting, R. Y. C. *J. Biol. Chem.* **1987**, *262*, 2187.
6. (a) Perini, F.; Carey, F. A.; Long, L. Jr. *Carbohydr. Res.* **1969**, *11*, 159. (b) Bamford, M. J.; Humber, D. C.; Storer, R. *Tetrahedron Lett.* **1991**, *32*, 271. (c) Sells, T. B.; Nair, V. *Tetrahedron* **1994**, *50*, 117. (d) Hammerschmidt, F.; Polsterer, J.; Zbiral, E. *Synthesis* **1995**, 415. (e) Janes, L. E.; Cimpoia, A.; Kazlauskus, R. J. *J. Org. Chem.* **1999**, *64*, 9019.
7. Ahn, S. K.; Kim, D.; Son, H. J.; Jeong, B. S.; Hong, R. K.; Kim, B. Y.; Kim, E. N.; Chung, K. H.; Kim, J. W. *J. Chem. Soc., Chem. Commun.* **1998**, 967.
8. (a) Faber, K. *Biotransformations in Organic Chemistry*; Springer: Berlin, 1997. (b) Klibanov, A. M. *Enzymatic Reactions in Organic Media*; Blackie Academic: Glasgow, 1996. (c) Davies, H. G. *Biotransformations in Preparative Organic Chemistry*; Academic Press: London, 1989.
9. Ahn, S. K.; Son, H. J. (Chong Kun Dang Pharmaceutical Co.) unpublished results; L-form $[\alpha]^{20}_D$ = +48.6 (0.17, MeOH), D-form $[\alpha]^{20}_D$ = –44.4 (0.18, MeOH).