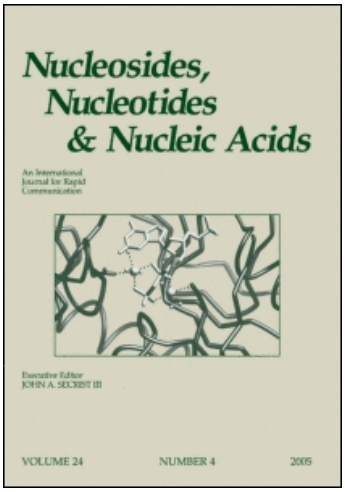

Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Ozaki, Shoichiro , Yamashita, Kayoko , Konishi, Takako , Maekawa, Takahiro , Eshima, Mitsuaki , Uemura, Atsuliiko and Ling, Lei(1995) 'Enzyme Aided Regioselective Acylation of Nucleosides', Nucleosides, Nucleotides and Nucleic Acids, 14: 3, 401 — 404

To link to this Article: DOI: 10.1080/15257779508012394

URL: http://dx.doi.org/10.1080/15257779508012394

NUCLEOSIDES & NUCLEOTIDES, 14(3-5), 401-404 (1995)

ENZYME AIDED REGIOSELECTIVE ACYLATION OF NUCLEOSIDES

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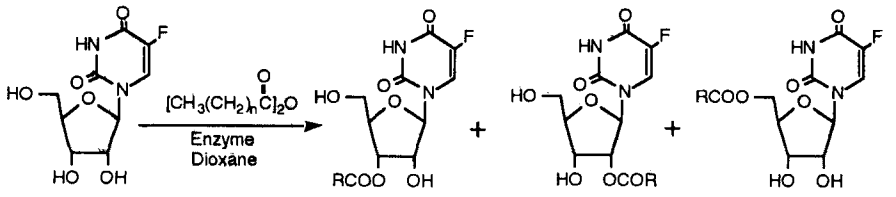
ABSTRACT : Selective protection of the three hydroxyl groups of sugar moiety of nucleosides have been studied by enzyme-catalyzed esterification in organic solvents. Selectively protected products were obtained. The reaction provides an efficient method for selective protection of nucleosides.

Introduction

Nucleoside derivatives are essential targets for the development of new medicines. To decrease the toxicities, and to improve the activities, chemical modifications, including protection, of nucleosides have been studied. Selective protection of the hydroxyl groups of the sugar moiety of nucleosides is useful for oligonucleotide synthesis.

Lipase catalyzed esterifications in organic solvents have been well-documented and proved to be a successful method [1-3]. Selective esterification of the hydroxyl groups of 2'-deoxyribonucleosides has been reported with high regioselectivity [4-6]. In the case of ribonucleosides, the primary hydroxyl group was selectively acylated [7]. In our study on chemical synthesis of biologically interesting 5-fluorouridine derivatives, we have applied this method for selective acylation of the multiple hydroxyl groups. We would like to report that the hydroxyl groups at C-2', C-3' and C-5' (2'-OH, 3'-OH and 5'-OH) of 5-fluorouridine (FUR) 1 were acylated in a highly regioselective manner (Scheme 1, Table 1).



SCHEME 1

TABLE 1 : Enzymatic Acylation of FUR **1** with n-Octanoic Anhydride

Enzyme (10 mass eq)	Reaction Time (h)	Conv.(%) *	Yield(%) 2	3	4
Lipase PS	30	100	92	1	trace
KWI-56	3	98	86	2	4
SP435	10	100	trace	1	90
Lipase M	95	50	3	42	-

n-Octanoic anhydride : 3 eq., $R=CH_3(CH_2)_6$ * Total yield of acylated products

Results and Discussion

Selective acylations of **1** using enzymes are shown in Table 1. When Lipase PS (a lipase from *Pseudomonas sp.*, Amano) and KWI-56 (a lipase from *Psudomonas sp.*, Kurita water industries ltd.) were used, the 3'-OH was acylated almost exclusively to give **2**. Lipase M (a lipase from *mucor javanicus*, Amano) acylated the 2'-OH to give **3**. SP435 (a lipase from *Candida antarctica*, Novo Nordisk) acylated the 5'-OH to give **4**. Only a small amount of diacylated and triacylated products were detected in all the cases. The proceeding of reaction was analyzed by HPLC. The migration of acyl group from C-3' position to C-2' position was observed during chromatography [8-11].

Apolar organic solvents, like a diethyl ether, are usually used in enzymatic esterification, but FUR is insoluble in these solvents. 1,4-Dioxane, THF, acetonitorile, DMF and acetone were tried, and dioxane and THF were found to be good solvents for this reaction.

We investigated the effect of chain length of alkyl carboxylic acid anhydride as acylation reagents. As shown in Table 2, the acylation with n-octanoic anhydride showed higher selectivity. In this regard, the acyl group with long alkyl chain showed higher stability.

Selective protection of uridine and arabinosyluracil were investigated,as shown in Table 3. The acylation of these compounds proceeded faster than the acylation of FUR under the same conditions. As shown for FUR, 3'-OH of uridine was acylated by Lipase PS and KWI-56, and the 5'-OH was acylated by SP435. The 5'-OH of arabinosyluracil was selectively protected by SP435.

We also attempted at recycling the enzymes. After the reaction, enzymes were filtrated, washed with MeOH and dried under vacuum. The recovered enzyme was almost inactive (Table 4). To improve the activity, we considered hydration of the enzyme according to the references [1,12]. A certain amount of water was added to soak the recovered enzyme. After the excess of water had been evaporated, the enzyme was dried under vacuum and reused. The recovered enzyme treated in this way showed almost the same activity as the fresh enzyme (Table 4). Thus, the enzyme was reactivated by hydration, indicating the importance of hydration for enzyme activity in an anhydrous organic solvent.

TABLE 2 : The Effect of Chain Length of Alkyl Carboxylic Acid Anhydrides

Acid anhydride (3 eq.)	Enzyme (10 mass eq)	Reaction Time (h)	Conv.(%)	Yield(%)		
				C-3'	C-2'	C-5'
$(CH_3CO)_2O$*	Lipase PS	23	98	68	1	16
$[CH_3(CH_2)_4CO]_2O$	Lipase PS	24	90	67	13	4
	KWI-5 6	23	88	76	5	trace
	SP435	8	94	1	-	85
$[CH_3(CH_2)_6CO]_2O$	Lipase PS	30	100	92	1	trace
	KWI-5 6	3	98	86	2	-
	SP435	10	100	trace	1	90
$[CH_3(CH_2)_{10}CO]_2O$	Lipase PS	72	81	69	5	2
	KWI-5 6	7	89	73	trace	9
	SP435	5	93	3	-	80

*Acetic acid anhydride : 6 eq. , Enzyme : 6 mass eq.

TABLE 3 : Esterification of Nucleosides with n-Caproic Anhydride

Nucleoside	Enzyme (10 mass eq)	Reaction Time (h)	Conv.(%)	Yield(%)		
				C-3'	C-2'	C-5'
5-Fluorouridine	Lipase PS	24	90	67	13	4
	KWI-56	23	88	76	5	trace
	SP435	8	94	1	-	85
Uridine	Lipase PS	8	100	83	9	-
	KWI-56	3	100	79	-	-
	SP435	5	100	trace	trace	96
Arabinosyluracil	SP435	3	87	3	-	77

n-Caproic Anhydride (3 eq.) was used in all reaction.

TABLE 4 : Activity of the Recovered Enzyme (Lipase PS)

Entry	Treatment before reuse of enzyme	Time(h)	Conv. (%)	Yield(%)		
				C-3'	C-2'	C-5'
1	Washed with methanol	24	51	29	11	trace
2	Washed with methanol, then drenched with water	24	91	80	3	2
*	Fresh enzyme	24	100	84	4	trace

n-Caproic Anhydride : 10 eq ; Enzyme : 3 mass eq

Experimental

To a solution of 5-fluorouridine (15.10mg, 0.06mmol) in anhydrous 1,4-dioxane (2mL) was added enzyme powder (Lipase PS, 157.38mg) and octanoic anhydride (55μl, 0.19mmol). The mixture was stirred at room temperature. The proceeding of reaction was checked and the yields of products were analyzed by HPLC (silica gel column, hexane/AcOEt/CH2Cl2/MeOH=30:5:5:3). The enzyme was filtered off when the reaction reached the expected point.

Acknowledgments

We thank Amano Pharmaceutical Co. Ltd., Kurita Water Industries Ltd. and Novo Nordisk Bioindustry Ltd. for kind supply of the enzymes. This work was partly supported by Grant in Aid for Scientific Research from the Ministry of Education, Science and Culture in Japan.

REFERENCES

1. Chen, C. S.; Sih, C. J. *Angew. Chem., Int. Ed. Engl.*, **1989**, *28*, 695.
2. Klibanov, A. M. *Acc. Chem. Res.*, **1990**, *23*, 114.
3. Wang, Y. F.; Lalonde, J. J.; Momongan, M.; Bergbreiter, D. E.; Wong, C. H. *J. Am. Chem. Soc.*, **1988**, *110*, 7072.
4. Moris, F.; Gotor, V. *J. Org. Chem.*, **1992**, *57*, 2490.
5. Nozaki, K.; Uemura, A.; Yamashita, J.; Yasumoto, M. *Tetrahedron Lett.*, **1990**, *31*, 7327.
6. Uemura, A.; Nozaki, K.; Yamashita, J.; Yasumoto, M. *ibid.*, **1989**, *30*, 3817.
7. Moris, F.; Gotor, V. *J. Org. Chem.*, **1993**, *58*, 653.
8. Green, D. P. L.; Reese, C. B. *Chem. Commun.*, **1968**, 729.
9. Baker, J.; Jarman, M.; Stock, J. A. *J. Chem. Soc., Perkin Trans.*, **1973**, *1*, 665.
10. Kempe, T.; Chow, F.; Sundquist, W. I.; Nardi, T. J.; Paulson, B.; Peterson, S. M. *Nucleic Acids Res.*, **1982**, *10*, 6695.
11. Wang, Y.; Chen, Y.-Q. *Heterocycles*, **1982**, *28*, 593.
12. Gupta, M. N. *Eur. J. Biochem.*, **1992**, *203*, 25-32.