Tetrahedron Letters,Vol.30,No.29,pp 3819-3820,1989 0040-4039/89 $3.00 + .00
Printed in Great Britain Maxwell Pergamon Macmillan plc

REGIOSELECTIVE DEPROTECTION OF 3',5'-O-ACYLATED PYRIMIDINE NUCLEOSIDES BY LIPASE AND ESTERASE

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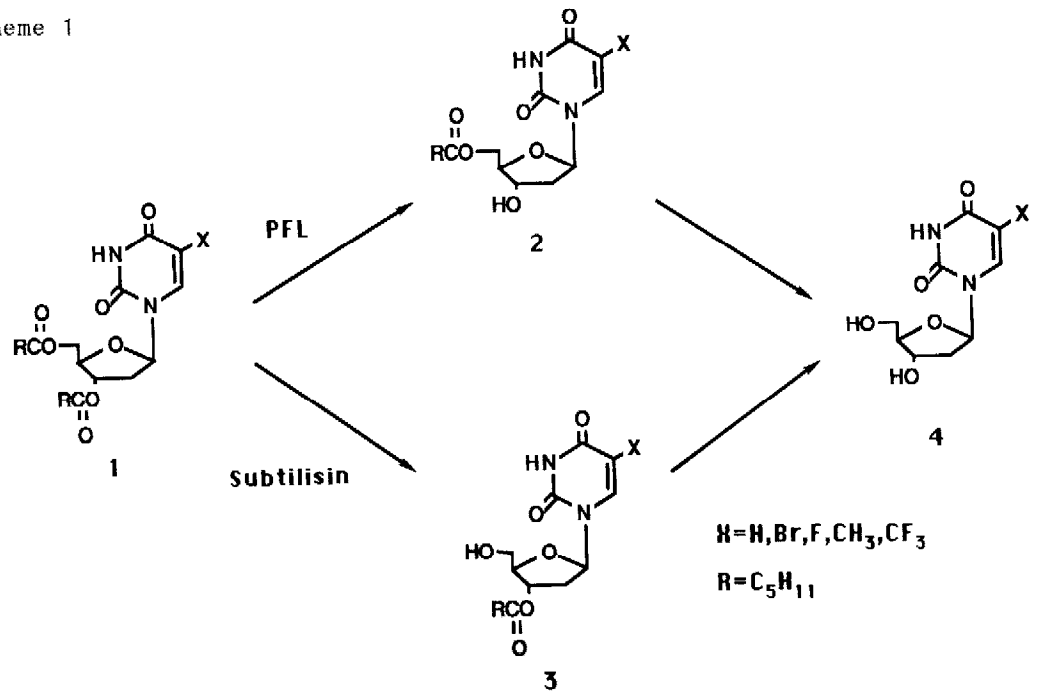
Abstract; A lipase was found to catalyze the regioselective hydrolysis at the secondary hydroxyl group of 2'-deoxy-3',5'-di-O-hexanoyl pyrimidine nuclosides, whereas a protease catalyzes that at the primary hydroxyl group.

Regioselective acylation and deacylation of sugars and related compounds mediated by enzymes have been developed as a useful synthetic method in organic reaction.[1-4] However, there is no report on regioselective deprotection of 3',5'-di-O-acylated nucleosides.

We found a simple and convenient procedure to obtain 2'-deoxy-5'-O-acylnucleoside (2) and 2'-deoxy-3'-O-acylnucleoside (3) by the hydrolysis of 2'-deoxy-3',5'-di-O-acylnucleoside (1) with a lipase from *Pseudomonas fluorescens* (PFL) and a protease from *Bacillus subtilis* (subtilisin), respectively (Scheme 1).

Scheme 1



In a typical run, 2'-deoxy-3',5'-di-O-hexanoyluridine (1, X = H)[5] (0.1 mmol) in dimethylformamide (DMF, 0.2 ml) and PFL (50 mg) were added to 0.1 N phosphate buffer (1.6 ml; pH 7), then the mixture was stirred at 25 °C. The reaction was monitored on TLC and HPLC. After 18 hr, the products were isolated by extracting the reaction mixture with ethyl acetate, drying, and evapolating the solvent. The yield were determined on HPLC. The reaction mixture was subjected to column chromatography on silica gel to afford 2'-deoxy-5'-O-hexanoyluridine (2, X = H; 71% yield)[6] together with 2'-deoxyuridine (4; 22% yield). The same procedure with subtilisin affords 2'-deoxy-3'-O-hexanoyluridine (3, X = H)[7] instead of 2, although the selectivity is not satisfactory (Table 1).

The present reactions proved a method for regioselective deacylation at the 5'- or 3'-position of nucleosides.

Table 1. Enzymatic Hydrolysis of 3',5'-Di-O-hexanoylpyrimidine Nucleosides[a]

X	Enzyme	Conv. (%)	Yield (%)[b] 1	2	3	4
H	PFL	93	7	71	0	22
H	Subtilisin	76	24	0	31	45
Br	PFL	100	0	80	0	20
Br	Subtilisin	68	32	0	12	54
F	PFL	100	0	74	0	26
F	Subtilisin	93	7	0	22	71
CH_3	PFL	100	0	58	0	42
CH_3	Subtilisin	93	7	0	28	65
CF_3	PFL	68	32	62	0	6
CF_3	Subtilisin	2	98	1	1	0

[a] All reactions were run at 25 °C for 18 hr with stirring.
[b] Yields were determined on HPLC (Reverse phase column ODS 1HU from Mitsubisi Chemical Ltd.; Mobil phase, CH_3CN : H_2O = 7 : 3 v/v).

Acknowledgement. We thank Amano Seiyaku Co., Ltd. for the supply of enzymes.

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5) Several diacylated 2'-deoxyuridines were subjected to the hydrolysis and it was found that the dipentanoyl and dioctanoyl derivatives are as good substrates as the dihexanoyl derivative, but the diacetyl derivative is not suitable for this purpose.
6) ^{1}HNMR(DMSO-d_6) δ 0.95 (3H, s), 1.00 - 1.70 (6H,m), 2.00 - 2.30 (4H, m), 3.80 - 4.40 (4H, m), 5.35 (1H, d), 5.65 (1H, d), 6.15 (1H, t), 7.62 (1H, d), and 11.40 (1H, s).
7) ^{1}HNMR(DMSO-d_6) δ 0.92 (3H,s), 1.00 - 1.70 (6H, m), 2.00 - 2.40 (4H, m), 3.50 - 3.65 (3H, m), 5.35 (1H, bs), 5.20 (1H, bs), 5.65 (1H, d), 6.12 (1H, t), and 11.30 (1H, s).

(Received in Japan 26 April 1989)