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Novozym 435-catalyzed regioselective acylation of 1-β-D-arabinofuranosylcytosine in a co-solvent mixture of pyridine and isopropyl ether

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Abstract

Novozym 435-catalyzed regioselective acylation of 1-β-D-arabinofuranosylcytosine (ara-C) for the preparation of its 5′-*O*-acyl derivative has been successfully performed for the first time. The reaction was dramatically accelerated by using vinyl acetate as the acyl donor and the co-solvent mixture of pyridine and isopropyl ether (3/1, v/v) as the reaction medium. Comparative study showed a lower E_a for the reaction taking place in the co-solvent mixture of pyridine and isopropyl ether (3/1, v/v) than in other media tested. The most suitable initial water activity, 1-β-D-arabinofuranosylcytosine concentration, molar ratio of vinyl acetate to 1-β-D-arabinofuranosylcytosine and reaction temperature were 0.11, 40 mM, 15:1 and 50 °C, respectively, under which the substrate conversion and the regioselectivity were as high as >97 and >99%, respectively, after a reaction time of 12 h.

Keywords: Enzymatic acylation; Regioselectivity; 1-β-D-Arabinofuranosylcytosine; Vinyl acetate; Co-solvent mixture

1. Introduction

Regioselective acylation of polyhydroxy compounds is a fundamental challenge to organic chemists. In the case of nucleosides, it is of particular importance as successful developments in this area may lead to new methods for introducing protecting groups [1,2]. On the other hand, regioselective acylation of nucleosides represents a way of obtaining nucleoside derivatives of high significance in medicinal chemistry. It has been proved that even just a single acylation can lead to compounds with higher antitumor activity, such as 5′-*O*-acyl-1-β-D-arabinofuranosylcytosine, which shows higher bioactivity than 1-β-D-arabinofuranosylcytosine (ara-C) itself [3,4].

Several strategies for selective acylation of ara-C have been reported employing conventional chemical methods, but their application is somewhat hampered by the relatively low regioselectivity, the lack of easy access to some of the key intermediates, the tedious product isolation and the environmental concerns of the process [1,4–6].

Recently, enzymes, such as lipases and proteases have proved to be effective catalysts for highly regioselective reactions [1]. Riva et al. [7] reported the protease-catalyzed acylation of adenosine and uridine with trichloroethyl butyrate in anhydrous DMF, gaining the desired 5′-*O*-acyl derivatives with moderate regioselectivity (55–85%). Subsequently, many studies on the acylation of nucleosides, employing activated acylating reagents (such as enol esters, acid anhydrides or oxime derivatives) catalyzed by protease or lipase have been reported. For example, Wong et al. [8] have reported the 5′-*O*-monoacylation of some nucleosides (not including ara-C) using a subtilisin mutant in DMF. Proteases showed rather inflexible selectivity towards the 5′-hydroxyl group of nucleosides [1,7,8], except for the case of adenosine, which could be esterified at 3′-hydroxyl group in a co-solvent mixture of DMSO and DMF [9]. Lipases seemed to be more attractive biocatalysts for this purpose as they showed high regioselectivity (up to 99% in most cases reported) and could catalyze the acylation of nucleosides at various hydroxyl groups depending on their sources and the reaction media used [1,10,11]. However, there have been few

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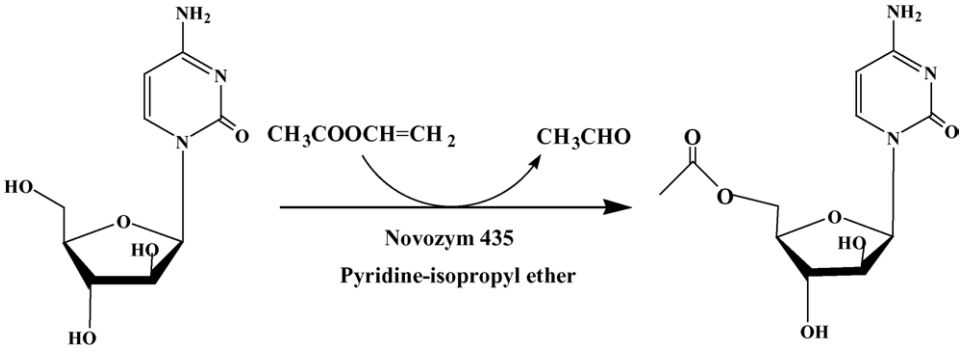

Fig. 1. Lipase-catalyzed regioselective acylation of 1-β-D-arabinofuranosylcytosine with vinyl acetate.

reports on the enzymatic acylation of ara-C because cytosine nucleosides are regarded as unreactive compared with other nucleosides [1,2] although Morís and Gotor [12] described the lipase-catalyzed 5′-*O*-monoacylation of *N*-butyryl-1-β-D-arabinofuranosylcytosine.

In our ongoing research related to the synthesis of lipophilic derivatives of ara-C, Novozym 435 (an immobilized lipase from *Candida antarctica*, type B) has been found to be highly regioselective towards the 5′-hydroxyl group of ara-C. Here we describe the enzymatic acylation of ara-C with enol ester (vinyl acetate) in a co-solvent mixture (Fig. 1). To our knowledge, this is the first report of the enzymatic 5′-*O*-monoacylation of unmodified ara-C.

2. Materials and methods

2.1. Biological and chemical materials

Novozym 435 (immobilized lipase from *C. antarctica*, type B, 164 U/g, one unit corresponds to the amount of enzyme that produces 1 μmol of methyl oleate from triacylglycerol per minute at pH 7.0 at 35 °C) was a generous gift from Novozymes (Denmark). 1-β-D-Arabinofuranosylcytosine and Reichardt's dye were purchased from Sigma (USA). All other chemicals were from commercial sources and were of the highest purity available.

2.2. General procedure for enzymatic acylation of ara-C

In a typical experiment, 2 ml of co-solvent mixture of pyridine and isopropyl ether containing 9.7 mg (0.04 mmol) ara-C, 55.0 μl (0.6 mmol) vinyl acetate and 16.4 U (100.0 mg) Novozym 435 was incubated in a 10 ml Erlenmeyer shaking-flask capped with a septum at 250 rpm and 30 °C. Aliquots were withdrawn at specified time intervals from the reaction mixture, and then diluted by 100 times with a co-solvent mixture of water and methanol prior to HPLC analysis. In order to structurally characterize the product, the reaction was scaled up (∼50 mg ara-C and 283 μl vinyl acetate). Upon the completion of the reaction, the reaction mixture was filtered to remove the immobilized enzyme and evaporated under vacuum. The crude product was then purified by silica gel chromatography with the mixture of methanol and chloroform (25/75, v/v) as an eluant. After crystallization from ethanol, the product was obtained as a white powder (53 mg, yield > 90%).

2.3. HPLC analysis

The reaction mixture was analyzed by RP-HPLC on a 4.6 mm × 250 mm, 5 μm Zorbax SB-C18 column (Agilent Technologies Co. Ltd., USA) using an Agilent G1311A pump and a UV Detector at 276 nm. The mobile phase was a mixture of ammonium acetate buffer (0.01 M, pH 4.27) and methanol (82/18, v/v) at a flow rate of 0.9 ml/min. The retention times for 1-β-D-arabinofuranosylcytosine and 5′-*O*-acetyl-1-β-D-arabinofuranosylcytosine were 3.22 and 9.32 min, respectively. Regioselectivity was defined as the ratio of the HPLC peak area corresponding to the indicated main product to that of all the products formed [13]. The initial rate (V_0) and the substrate conversion (c) were calculated from the HPLC data. The average error for this determination is less than 0.7%. All reported data are averages of experiments performed at least in duplicate.

2.4. Structure determination

The position of acylation in enzymatically prepared ester was determined by ^{13}C NMR and ^{1}H NMR (Bruker AVANCE Digital 400 MHz Nuclear Magnetic Resonance Spectrometer, Bruker Co., Germany) at 75 and 300 MHz, respectively. DMSO-d_6 was used as solvent and chemical shifts were expressed in ppm shift. The FT-IR spectra of the product were recorded on a Nicolet NEXUS™ 470 Fourier-Transform Infrared Spectrometer (Thermo Nicolet Co., USA).

2.5. Control of the initial water activity

The initial water activities of the reaction medium, the substrate and the enzyme were controlled by gaseous equilibrium with different saturated salt solutions in separate closed containers at 25 °C. The following salts were used: LiCl ($a_w = 0.11$), $MgCl_2$ ($a_w = 0.33$), $Mg(NO_3)_2$ ($a_w = 0.53$), NaCl ($a_w = 0.75$), KCl ($a_w = 0.85$) and K_2SO_4 ($a_w = 0.97$) [14–18]. Molecular sieve was used to generate the nearly anhydrous reaction medium ($a_w \sim 0$). For the reaction medium, the equilibration was mon-

itored by water analysis using Karl Fischer titration with a 787 KF Titrino (Metrohm Co. Ltd., Switzerland).

2.6. Polarity parameter $E_T(30)$ determination for pure and binary mixtures of solvents

1.5 mg of Reichardt's dye was dissolved in 100 ml of either a pure solvent or a two-solvent mixture. Then, the maximal UV–vis absorption band of this dye was recorded by a UNIICO™ WFZUV-2102 PC spectrophotometer (UNICO instruments Co. Ltd., China). The polarity of the reaction medium was estimated using the empirical parameter $E_T(30)$ described by Reichardt [19] and Castillo et al. [20] and $E_T(30)$ was calculated from the equation:

$$E_T(30)\,(\text{kcal/mol}) = h\nu N = h\left(\frac{c}{\lambda}\right)N \quad (1)$$

where h is the Planck's constant, ν the light frequency, c the velocity of light, λ the maximal UV–vis absorption band of Reichardt's dye and N is the Avogadro's number. The result is reported in Table 3.

2.7. Determination of activation energy

The reaction was performed in various reaction media (pure solvent or co-solvent mixture) and different temperatures (varying from 25 to 50 °C). 16.4 U (100.0 mg) enzyme was added to 2 ml of the reaction mixture ($a_w = 0.11$) containing 9.7 mg (0.04 mmol) ara-C and 55.0 μl (0.6 mmol) vinyl acetate. The mixture was then incubated in a water-bath shaker at 250 rpm. The apparent activation energy (E_a) of Novozym 435-catalyzed acylation of ara-C with vinyl acetate was calculated according to the Arrhenius equation.

3. Results and discussion

3.1. Regioselectivity of the reaction

The ability of Novozym 435 to catalyze regioselective transformation has been extensively exploited in the modification of polyhydroxy compounds [21,22]. In our detailed examination of its catalysis of the acylation of ara-C with vinyl acetate in a co-solvent mixture of isopropyl ether and pyridine, Novozym 435 exhibits startling regioselectivity up to 99% towards the 5′-hydroxyl group of ara-C.

The structure of the product was determined on the basis of its spectral data. According to Yoshimoto et al. [23], the acylation of a hydroxyl group of sugar results in a downfield shift of the peak corresponding to the *O*-acylated carbon atom and an upfield shift of the peak corresponding to the neighboring carbon atom. This is confirmed by the data listed in Table 1, where the ^{13}C NMR spectrum of the product shows a calculated shift of 3 ppm on C5′ towards the lower fields as compared to the same carbon atom in the unmodified ara-C, and the datum of the directly neighboring carbon atom, C4′, also changes by approximately 3 ppm due to its effect. In addition, two sharp peaks of $-CH_3$ and C=O appear with the determinate chemical shifts.

Table 1
^{13}C NMR spectral data for ara-C and its acylated derivative (δ, ppm)[a]

Carbon numbers	ara-C	5′-*O*-Acetyl ara-C
Base moiety		
2	154.25	154.24
4	164.86	165.12
5	93.07	93.23
6	144.02	143.86
Sugar moiety		
1′	86.49	86.73
2′	75.13	74.81
3′	76.64	77.13
4′	85.52	82.64
5′	61.63	64.33
Acyl moiety		
C=O		170.74
$-CH_3$		21.11

[a] All samples measured in DMSO-d_6.

The position of the acylation was furthermore confirmed by the ^{1}H NMR and IR spectral data [^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 7.51 (1H, d, H-6), 7.43 (2H, br d, NH_2), 6.05 (1H, d, H-5), 5.75 (1H, d, H-1′), 5.57–5.41 (2H, m, OH-2′ and OH-3′), 4.26 (1H, m, H-5′$_1$), 4.12 (1H, m, H-5′$_2$), 3.88–3.55 (3H, m, H-2′, H-3′ and H-4′), 2.00 (3H, d, CH_3); FT-IR (KBr, cm^{-1}): 3340 (OH), 3207 (NH), 1723 (C=O)].

Novozym 435-catalyzed acylation of ara-C with vinyl acetate proceeds much faster than the cases with acetic acid or ethyl acetate as the acyl donor, as the formed enol can rapidly tautomerize to the corresponding volatile aldehyde, avoiding the back reaction and making the reaction irreversible. Besides, the chemical acylation reaction did not take place in the absence of the enzyme under the reaction conditions tested. In addition, acid anhydride has been proved unsuitable for the preparation of 5′-*O*-monoester of ara-C because it causes chemical acylation at the N^4 position, giving *N*-*O*-acylated compound.

3.2. Effect of initial water activity

Generally speaking, water activity (a_w) plays a crucial role in enzymatic reactions in nonaqueous media [14–18,24]. It is of great significance to pay attention to water control since the presence of water may foster the competitive hydrolysis of both 5′-*O*-acyl ara-C and vinyl acetate.

As shown in Table 2, Novozym 435-mediated acylation of ara-C shows a clear a_w dependence in the pyridine–isopropyl ether co-solvent system. Both the initial reaction rate and the substrate conversion increased rapidly and reached maximum at $a_w = 0.11$, while rather low reaction rate and substrate conversion were observed when the reaction was carried out in medium with lower or especially higher a_w values. This is because the presence of water in the reaction medium is essential for the enzyme to keep its catalytic conformation. On the other hand, water promoted the side hydrolysis reactions of both the product and the acyl donor. The optimal water activity represented the most appropriate water condition from the balance between these conflicts, whereas the lower water activity provide insuf-

Table 2
Effect of initial water activity on the enzymatic acylation of ara-C

a_w	V_0 (mM/h)	C (%)[a]	Regioselectivity (%)
~0	57.2	80.5	>99
0.11	73.1	97.6	>99
0.33	39.9	69.6	>99
0.53	17.7	10.1	>99
0.75	9.0	7.3	>99
0.85	8.8	7.1	>99
0.97	8.0	6.8	>99

The reactions were initiated by adding 16.4 U (100.0 mg) Novozym 435 into a 2 ml of solvent mixture (with water activity varying from 0.11 to 0.97) of pyridine and isopropyl ether containing 9.7 mg (0.04 mmol) ara-C and 55.0 μl (0.6 mmol) vinyl acetate and performed at 30 °C, 250 rpm.
[a] Maximum conversion of the reaction.

ficient water for the buildup of the essential water shell for enzyme, and the higher water activity implied excessive water and thereby the increasing competition of water for the hydrolysis of acyl–enzyme intermediate [25,26].

3.3. Effect of reaction medium

One of the most troublesome limitations in the acylation of hydrophilic nucleosides is their poor solubility in most organic solvents. In fact, only polar organic solvents, such as pyridine and DMF, have been commonly used [7,27]. However, the polar organic solvents usually strip the essential water off the enzyme molecules and then inactivate the biocatalyst, which greatly limits the application of enzymatic procedures in these areas. To characterize the polarity of the solvents, the parameter $E_T(30)$, which has been considered to be a good measure of the polarity for both pure solvent and co-solvent mixture [19,20], was introduced.

As can be seen in Table 3, among the pure solvents, the higher the polarity of the solvent, the lower the reaction rate and substrate conversion. This also applies to the cases with the solvent mixtures. The reaction proceeded with much higher rate and substrate conversion in the co-solvent mixture of tetrahydrofuran and pyridine (1/3) than in pyridine in spite of their similar polarity, suggesting that the polarity of the reaction medium is not the only factor influencing the reaction. In the mixture of isopropyl ether and pyridine (1/3, v/v) with the lowest $E_T(30)$ value (40.05 kcal/mol), both the highest initial rate (73.0 mM/h) and the highest conversion (97.6%) were achieved. This could be accounted for by the less inactivating effect of the less polar medium on the enzyme.

The shifting of reaction medium did not lead to substantial changes in the regioselectivity of the reaction. In most reaction media assayed, the regioselectivity of the reaction was higher than 98%.

3.4. Effect of ara-C concentration and the ratio of vinyl acetate to ara-C

Thermodynamically, high substrate concentration may push the reaction towards the product formation and speed up the reaction. Substrate inhibition, however, may occur when substrate concentration is excessively high.

As can be seen in Fig. 2a, when substrate concentration was below 40 mM, the initial rate of the acylation increased with increasing substrate concentration. Further increase in substrate concentration led to little change in reaction rate. Within the scope examined, no substrate inhibition was observed. Although the maximal conversion of ara-C was hardly effected by varying the substrate concentration, much longer time was needed for the reaction to reach the maximum conversion with higher substrate concentration.

When ara-C concentration was fixed at 20 mM, the enzymatic acylation of ara-C was greatly affected by the ratio of vinyl acetate to ara-C. As shown in Fig. 2b, remarkable enhancement in both the initial rate and the substrate conversion was observed with the increase of the ratio up to 15, which is the optimal ratio of vinyl acetate to ara-C. The excessive amount of vinyl acetate was necessary for lipase-catalyzed acylation, which was in good accord with the recent report [11]. It has been proved experimentally that the presence of excessive amount of vinyl acetate inhibits the hydrolysis of the product (5′-*O*-acetyl ara-C) and pushes the reaction equilibrium towards the synthesis of 5′-*O*-acetyl ara-C. Additionally, the hydrolysis of vinyl acetate

Table 3
Effect of reaction medium on the reaction

Solvent[a]	$E_T(30)$ (kcal/mol)	V_0 (mM/h)	C (%)[b]	Regioselectivity (%)
DMSO	45.10	0	0	0
DMF	43.50	2.7	5.9	>99
Pyridine	40.20	22.0	43.4	>99
Acetonitrile–pyridine (1:3)	42.89	17.3	45.8	>99
t-Butanol–pyridine (1:3)	42.01	20.4	49.1	98
t-Pentyl alcohol–pyridine (1:3)	41.88	36.3	67.4	98
Tetrahydrofuran–pyridine (1:3)	40.17	43.0	83.6	>99
Hexane–pyridine (1:3)	40.06	72.8	97.0	99
Isopropyl ether–pyridine (1:3)	40.05	73.0	97.6	>99

The reactions were initiated by adding 16.4 U (100.0 mg) Novozym 435 into a 2 ml of pure solvent or solvent mixture (a_w = 0.11) containing 9.7 mg (0.04 mmol) ara-C and 55.0 μl (0.6 mmol) vinyl acetate and performed at 30 °C, 250 rpm.
[a] Mixtures of solvents (v/v).
[b] After a reaction time of 12 h.

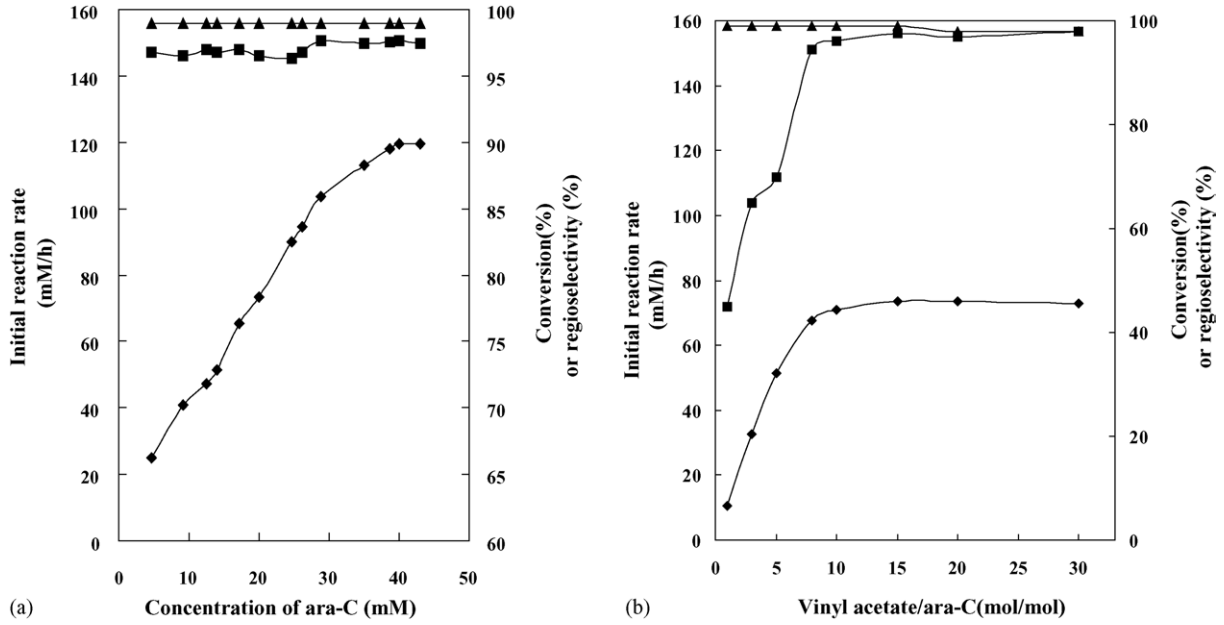

Fig. 2. (a) Effect of ara-C concentration on the reaction. The reactions were carried out at 30 °C, 250 rpm by adding 16.4 U (100.0 mg) Novozym 435 into 2 ml of co-solvent mixture of pyridine and isopropyl ether (3/1, v/v) containing different amounts of ara-C and 55.0 μl (0.6 mmol) vinyl acetate with controlled initial water activity (a_w = 0.11). *Symbols*: (♦) initial reaction rate; (■) maximum conversion of ara-C and (▲) regioselectivity. (b) Effect of the ratio of vinyl acetate to ara-C on the reaction. The reactions were performed in 2 ml of co-solvent mixture of isopropyl ether–pyridine (1/3, v/v) with controlled initial water activity (a_w = 0.11) at 30 °C, 250 rpm. The reaction mixture contained 9.7 mg (0.04 mmol) ara-C and various molar ratios of vinyl acetate to ara-C in the presence of Novozym 435 (16.4 U, 100.0 mg). *Symbols*: (♦) initial reaction rate; (■) maximum conversion of ara-C and (▲) regioselectivity.

might consume considerable amount of vinyl acetate and lower the acylation rate and substrate conversion [26].

It is also worth-noting that only a minor change in the regioselectivity of the reaction occurs with the change in either the concentration of ara-C or the ratio of vinyl acetate to ara-C.

3.5. Effect of reaction temperature

Temperature has great effect on the activity, selectivity and stability of a biocatalyst and the thermodynamic equilibrium of a reaction as well. As shown in Fig. 3, within the range from 25 to 50 °C, higher temperature resulted in both higher initial rate and higher substrate conversion. Further rise in temperature, however, led to a drastic drop in both the initial rate and the substrate conversion. The regioselectivity of the reaction remained above 99% at temperatures ranging from 20 to 60 °C. The serious volatilization of vinyl acetate and the partial inactivation of the lipase in pyridine–isopropyl ether mixture at high temperature above 50 °C may partly account for the drop in both the initial rate and the substrate conversion.

3.6. Activation energy of the reaction

To get a deeper insight into the effect of the co-solvent mixtures on Novozym 435-catalyzed acylation of ara-C, a comparative study on the activation energy of the enzymatic reaction was conducted in pure pyridine, *t*-pentyl alcohol–pyridine mixture (1/3, v/v) or isopropyl ether–pyridine (1/3, v/v), respectively. The values of E_a determined for this reaction are given in Table 4. As predicted, the E_a of the reaction performed in the co-solvent mixture of isopropyl ether and pyridine (1/3, v/v) was the lowest. The effect of reaction medium on the activation energy could well account for its influence on the reaction rate.

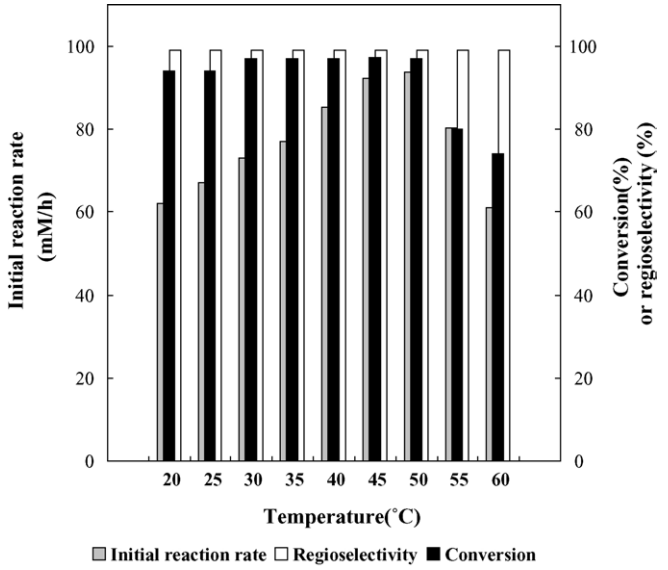

Fig. 3. Effect of reaction temperature on the reaction. The reactions were initiated by adding 16.4 U (100.0 mg) Novozym 435 to 2 ml of co-solvent mixture of pyridine and isopropyl ether (3/1, v/v; a_w = 0.11) containing 9.7 mg (0.04 mmol) ara-C and 55.0 μl (0.6 mmol) vinyl acetate and performed at 250 rpm and various temperatures from 20 to 60 °C (in 5 °C intervals).

Table 4
Comparision of E_a of Novozym 435-catalyzed acylation of ara-C in different reaction media

Solvent	E_a (kJ/mol)
Pyridine	30.1
t-Pentyl alcohol–pyridine (1/3, v/v)	24.4
Isopropyl ether–pyridine (1/3, v/v)	11.0

The reactions were carried out in 2 ml of the reaction media ($a_w = 0.11$) containing 9.7 mg (0.04 mmol) ara-C, 55.0 μl (0.6 mmol) vinyl acetate and 16.4 U (100.0 mg) enzyme at temperatures from 20 to 50 °C, 250 rpm.

4. Conclusions

The procedure described here represents a facile way to introduce protective groups in ara-C or to synthesize its novel derivatives with potential antitumor activities. In contrast to the multi-step chemical process, it is more promising because of its excellent regioselectivity, simplicity, environmental friendliness and mild reaction conditions. The findings of this research further highlight the versatility of lipases.

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References

[1] M. Ferrero, V. Gotor, Monatsh. Chem. 131 (2000) 585.
[2] F. Morís, V. Gotor, J. Org. Chem. 58 (1993) 653.
[3] A.M. Bergman, C.M. Kuiper, D.A. Voorn, E.M. Comijn, F. Myhren, M.L. Sandvold, H.R. Hendriks, G.J. Peters, Biochem. Pharmacol. 67 (2004) 503.
[4] D.T. Gish, R.C. Kelly, G.W. Camiener, W.J. Wechter, J. Med. Chem. 14 (1971) 1159.
[5] M. Kluge, H. Schott, US Patent 5,641,758 (June 24, 1997).
[6] F. Myhren, B. Børretzen, A. Dalen, K.T. Stokke, US Patent 6,335,322 B1 (January 1, 2002).
[7] S. Riva, J. Chopineau, A.P.G. Kieboom, A.M. Klibanov, J. Am. Chem. Soc. 110 (1988) 584.
[8] C.H. Wong, S.T. Chen, W.J. Hennen, J.A. Bibbs, Y.F. Wang, J.L.C. Liu, M.W. Pantoliano, M. Whitlow, P.N. Bryan, J. Am. Chem. Soc. 112 (1990) 945.
[9] Y. Tokiwa, M. Kitagawa, H. Fan, T. Yokochi, T. Raku, Y. Hiraguri, S. Shibatani, Y. Maekawa, N. Kashimura, R. Kurane, Biotechnol. Tech. 13 (1999) 563.
[10] P. Wang, J.S. Dordick, Macromolecules 31 (1998) 941.
[11] J. García, S. Fernández, M. Ferrero, Y.S. Sanghvi, V. Gotor, Tetrahedron Lett. 45 (2004) 1709.
[12] F. Morís, V. Gotor, Tetrahedron 49 (1993) 10089.
[13] M. Therisod, A.M. Klibanov, J. Am. Chem. Soc. 108 (1986) 5638.
[14] E. Wehtje, D. Costes, P. Adlercreutz, J. Mol. Catal. B: Enzym. 3 (1997) 221.
[15] P.J. Halling, Enzyme Microb. Technol. 16 (1994) 178.
[16] G. Bell, P.J. Halling, B.D. Moore, J. Partridge, D.G. Rees, Trends Biotechnol. 13 (1995) 468.
[17] A. Ducret, M. Trani, R. Lortie, Enzyme Microb. Technol. 22 (1998) 212.
[18] W.Y. Lou, M.H. Zong, H. Wu, R. Xua, J.F. Wang, Green Chem. 7 (2005) 500.
[19] C. Reichardt, Angew. Chem. Int. Ed. Engl. 18 (1979) 98.
[20] E. Castillo, F. Pezzotti, A. Navarro, A. López-Munguía, J. Biotechnol. 102 (2003) 251.
[21] E.M. Anderson, M. Karin, O. Kirk, Biocatal. Biotransform. 16 (1998) 181.
[22] R.W. McCabe, A. Taylor, Enzyme Microb. Technol. 35 (2004) 393.
[23] K. Yoshimoto, Y. Itatani, Y. Tsuda, Chem. Pharm. Bull. 28 (1980) 2065.
[24] A.M. Klibanov, Trends Biotechnol. 15 (1997) 97.
[25] P. Degn, W. Zimmermann, Biotechnol. Bioeng. 74 (2001) 483.
[26] H.K. Weber, H. Weber, R.J. Kazlauskas, Tetrahedron: Asymm. 10 (1999) 2635.
[27] Y.F. Wang, J.J. Lalonde, M. Momongan, D.E. Bergbreiter, C.H. Wong, J. Am. Chem. Soc. 110 (1988) 7200.