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Efficient synthesis of 5′-*O*-laurate of 1-β-D-arabinofuranosylcytosine via highly regioselective enzymatic acylation in binary solvent mixtures

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ABSTRACT

Regioselective enzymatic acylations of 1-β-D-arabinofuranosylcytosine (ara-C) with vinyl laurate (VL) in binary organic solvents were explored for the preparation of 5′-*O*-laurate of ara-C. Among the nine kinds of enzymes, Novozym 435 showed the highest regioselectivity (>99.9%) towards the 5′-OH of ara-C. This lipase showed higher catalytic activity in hexane–pyridine than in other tested solvent mixtures. The most suitable VL to ara-C molar ratio, initial water activity, and reaction temperature were shown to be 15:1, 0.07, and 50 °C, respectively, under which the initial reaction rate and the maximum substrate conversion were as high as 84.0 mmol L^{-1} h^{-1} and 98.1%, respectively. The product of Novozym 435-catalyzed acylation was characterized by ^{13}C NMR and confirmed to be 5′-*O*-laurate of ara-C.

1-β-D-Arabinofuranosylcytosine (ara-C) is a unique unnatural nucleoside with substantial antitumor, antiviral, and immunosuppressive effects. Especially, it has proved to be a valuable agent for the treatment of acute leukemias.[1,2] However, ara-C is hydrophilic and cannot easily traverse cell membranes by passive diffusion.[1] Besides, ara-C suffers rapid deamination and deactivation in vivo by cytidine deaminase. And its very short half-life (12 min) in human also restricts its clinical use.[2] A successful approach to increase its biological half-life was developed by work on the regioselective acylation of 5′-OH of ara-C with different acyl donors.[3,4] Certain of these achieved 5′-esters of ara-C showed dramatically altered antileukemic activities as compared to ara-C itself.

Conventional chemical methods for selective acylation of ara-C suffer from the relatively low regioselectivity, the lack of easy access to some key intermediates, and the environmental concerns of the process.[1–4] Enzymatic acylation of nucleosides in nonaqueous media has been proven to be a favorable and practicable option. In such a reaction the catalytic specificity of the enzyme would avoid the need for additional chemical protection/deprotection steps.[3,5,6]

In the past, we had successfully performed the enzymatic synthesis of short chain fatty acid esters of ara-C (such as 5′-acetyl ara-C) for development of a new method for introducing protecting groups.[7–10] It was found that compared with short chain fatty acid ester derivatives of ara-C, the long chain fatty acid ester derivatives have higher bioactivity but poorer reactivity due to steric hindrance.[1–3] For example, an increase in life span (expressed as% ILS) of 5′-laurate of ara-C was nearly 300%, which was much higher than that of 5′-acetyl ara-C (<25%).[2] However, there has been no report on enzymatic synthesis of acylation of ara-C with vinyl laurate (VL) so far. We herein for the first time describe the successful regioselective 5′-acylation of ara-C with VL by lipase in organic solvents. The effects of several crucial factors influencing the enzymatic acylation of ara-C were also systematically examined (Scheme 1). The lipase-catalyzed acylation of ara-C with VL might be a new route for the efficient synthesis of 5′-lauroyl ara-C, a derivative with dramatically enhanced activity than ara-C itself.

Due to the unparalleled positional selectivities, enzymes can be used in various transformations without tedious blocking and deblocking steps common in regioselective organic synthesis. Such a high selectivity affords the efficient reactions with few by-products.[3,5–10] One of the key point of present work was to determine the best catalyst for acylation of ara-C at 5′-hydroxyl positions. Therefore, nine potential lipases from various sources were screened for promising enzymes for the acylation of ara-C with VL and the products were structurally characterized by ^{13}C NMR (data expressed in References and notes section). As shown in Table 1, the highest V_0 (66.0 mmol L^{-1} h^{-1}) and conversion (96.0%) were achieved with Novozym 435 (from *Candida antarctica*) among all the enzymes assayed, which also showed high 5′-regioselectivity (>99.9%) and no 3′-ester product was produced by this lipase. The other lipases exhibited little or no activity for this reaction under the reaction conditions examined.

General enzymatic acylation procedure is illustrated in Scheme 1. Reactions were initialed by adding 500 U Novozym 435 into

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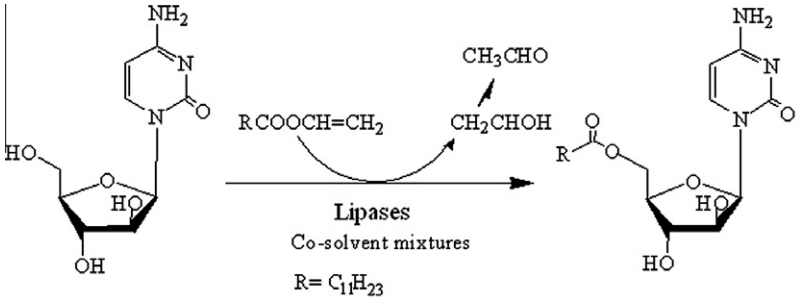

Scheme 1. Regioslective acylation of ara-C with VL by lipases in co-solvent mixtures.

Table 1
Acylation of ara-C with VL catalyzed by various enzymes

Enzyme	V_0 (mmol L^{-1} h^{-1})	C^b (%)	3′-Regiose-lectivity (%)	5′-Regiose-lectivity (%)
Novozym 435	66.0	96.0	~0	>99.9
Lipozyme IMTL	6.0	15.1	30.0	70.0
Lipozyme IMRM	6.5	15.3	26.0	74.0
Lipozyme IM	NA[a]	NA	NA	NA
CRL-VII	NA	NA	NA	NA
PPL	NA	NA	NA	NA
Lipase OF	NA	NA	NA	NA
Lipase A	NA	NA	NA	NA
Lipase M	NA	NA	NA	NA

[a] No activity.

1 mL of reaction media containing 0.02 mmol ara-C and 0.3 mmol VL, with shaking at a fixed temperature stated for each experiment. Aliquots were withdrawn at specified time intervals from the reaction mixture for HPLC analysis.[11] To structurally characterize the product of the Novozym 435-catalyzed acylation of ara-C with VL, the reaction was scaled up (0.08 mmol of ara-C and 2 mol VL). After filtration to remove the enzyme, reaction mixtures were evaporated under vacuum. The residue was extracted three times with ethanol and the collected solution was further evaporated to give the crude product. The vinyl ester derivatives obtained were characterized by ^{13}C NMR (Bruker AVANCE Digital 400 MHz).[12]

As the reaction medium had significant influences on both activity and selectivity of enzymes,[6–10] we performed the acylation of ara-C with VL in six kinds of co-solvent mixtures with different polarities (Table 2). As expected, the initial rate and the substrate conversion were well correlated with the polarity of the co-solvents. Within the $E_T(30)$ value of 42.89–40.05 kcal mol^{-1}, both the reaction rate and substrate conversion went up markedly with the decrease of $E_T(30)$ value. And hexane–pyridine co-solvent, with the lowest $E_T(30)$ value of 40.05 kcal mol^{-1}, gave the highest initial rate (70.2 mmol L^{-1} h^{-1}) and substrate conversion (97.5%). Results also indicated that the shift of the reaction medium barely affected the regioselectivity of the immobilized lipase in this reaction.

Weber et al. reported that, in the enzymatic acylation using vinyl esters as acyl donor, there existed a side reaction, that is, the hydrolysis of acyl donor.[13] Hence, an excess of acyl donor was normally necessary for effective enzymatic acylation of ara-C with VL. The results in Figure 1 confirmed that the substantial excess of VL was also necessary for Novozym 435-catalyzed acylation of ara-C with VL. Marked enhancements in both the initial reaction rate and the maximum substrate conversion were observed with an increase in the molar ratio up to 15:1, the optimal value of the molar

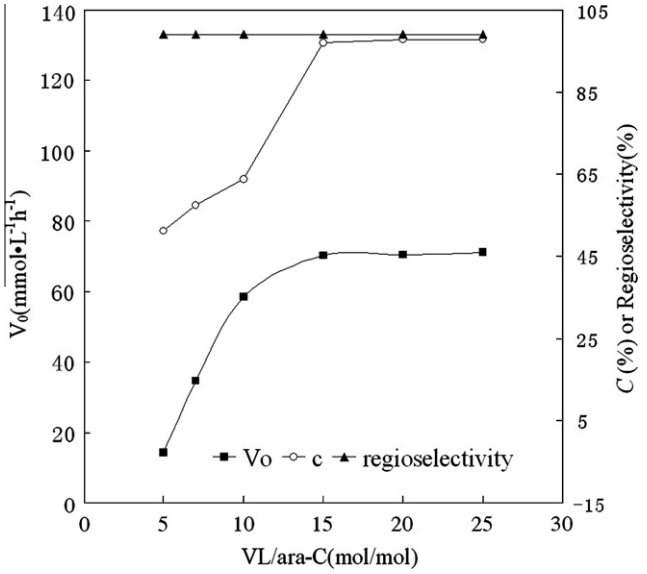

Figure 1. Effect of the molar ratio of VL to ara-C on the regioselective acylation of ara-C with VL mediated by Novozym 435 in hexane–pyridine.

Table 3
Effect of initial a_w on enzymatic acylation of ara-C with VL in hexane–pyridine

a_w	V_0 (mmol L^{-1} h^{-1})	C^b (%)	Regioselectivity (%)
~0	40.8	67.2	>99.9
0.07	70.2	97.6	>99.9
0.11	69.0	95.8	>99.9
0.33	14.2	10.7	>99.9
0.53	5.8	6.1	>99.9

Table 2
Effect of different pyridine-containing organic solvents on the enzymatic acylation of ara-C with VL

Solvent (v/v)	$E_T(30)$ (kcal mol^{-1})	V_0 (mmol L^{-1} h^{-1})	C (%)	5′-Regioselectivity (%)
Acetonitrile–pyridine (1:3)	42.89	18.3	31.8	>99.9
t-Butanol–pyridine (1:3)	42.01	21.4	35.1	>99.9
t-Pentyl alcohol–pyridine (1:3)	41.88	27.3	41.4	>99.9
Tetrahydrofuran–pyridine (1:3)	40.17	31.0	45.6	>99.9
Isopropyl ether–pyridine (1:3)	40.06	66.0	96.0	>99.9
Hexane–pyridine (1:3)	40.17	70.2	97.5	>99.9

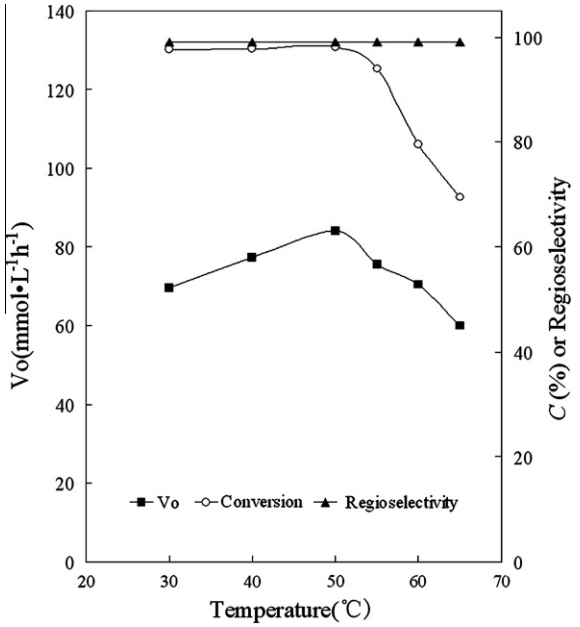

Figure 2. Effect of the reaction temperature on the regioselective acylation of ara-C with VL mediated by Novozym 435 in hexane–pyridine.

ratio of VL to ara-C, above which there was no appreciable increase in either initial rate or substrate conversion. It was also notable that only a minor change in the regioselectivity of the reaction occurred with the change in the ratio of the two substrates.

Since the presence of water may not only influence the active structure of lipase, but also promote the competitive hydrolysis of both the acylated ara-C and the vinyl esters, we paid attention to the effect of initial water activity (a_w) on the acylation reaction. Table 3 depicts that both the initial rate and substrate conversion increased with the increase of a_w till 0.07. Further increase in initial a_w, however, led to lower reaction rates and substrate conversion. Reaction temperature also showed obvious effect on the efficiency of the reaction (Fig. 2). The highest initial rate (84.0 mmol L^{-1} h^{-1}) and substrate conversion (98.1%) of Novozym 435-catalyzed acylation of ara-C in hexane–pyridine were achieved at the optimum temperature of 50 °C. As expected, temperature showed little effect on the regioselectivity within the range examined.

In summary, an efficient route for preparation of 5′-*O*-laurate of ara-C via highly regioselective acylation of ara-C with long chain vinyl ester was developed in this Letter. With this method, high substrate conversion (98.1%) were achieved, which was much higher than that using traditional chemical method (<72%). Besides, the one-step enzymatic approach is more promising due to its excellent 5′-regioselectivity (99.9%) and environmental friendliness, in contrast to the chemical methods which need tedious protection/deprotection procedures for a highly regioselctive lauroylation of ara-C. The novelty and importance of this work were, firstly, proving the feasibility and practicability of the 'enzymatic-solvent engineering' strategy for preparation of long chain fatty acid ester derivatives of nucleosides with high antitumor activities. This finding also gained more knowledge about the impact of nonaqueous media on catalytic behavior of lipase and further highlights the versatility of lipases for modulating enzymatic synthesis of nucleoside derivatives.

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11. *HPLC analysis:* Samples were analyzed by RP-HPLC on a 4.6 × 250 mm (5 μm) Zorbax SB-C18 column 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 (15/85, v/v) at a flow rate of 1.0 ml/min. The retention times for ara-C, 3′-*O*-lauroyl ara-C and 5′-*O*-lauroyl ara-C were 2.60 min, 6.28 min and 6.52 min, respectively.
12. ^{13}C NMR of Ara-C δ: 164.86 (C-4), 154.25 (C-2), 144.02 (C-6), 93.07 (C-5), 86.49 (C-1′), 85.52 (C-4′), 76.64 (C-3′), 75.13 (C-2′), 61.63 (C-5′); ^{13}C NMR of 5′-*O*-lauroyl ara-C. ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 173.29 (COO), 165.80 (C4–N), 155.62 (C=O 2), 143.37 (C-6), 93.35 (C-5), 86.97 (C-1′), 82.55 (C-4′), 77.27 (C-3′), 75.09 (C-2′), 64.16 (C-5′), 22.52–34.04 (CH_2), 13.81 (CH_3). ^{13}C NMR of 3′-*O*-lauroyl ara-C. ^{13}C NMR(DMSO-d_6, 100 MHz) δ: 172.36 (COO), 164.45 (C4–N), 155.85 (C=O 2), 144.46 (C-6), 93.49 (C-5), 86.90 (C-1′), 83.02 (C-4′), 79.16 (C-3′), 72.95 (C-2′), 61.42 (C-5′), 25.65–34.27 (CH_2), 14.48 (CH_3).
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