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Regioselective synthesis of 3′-*O*-caproyl-floxuridine catalyzed by *Pseudomonas cepacia* lipase

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Abstract

3′-*O*-Caproyl-floxuridine was prepared successfully through an enzymatic approach. Among the seven commercially available lipases tested, *Pseudomonas cepacia* lipase displayed the highest activity and regioselectivity towards the acylation of 3′-hydroxyl of floxuridine. Effects of various parameters on the acylation reaction catalyzed by *P. cepacia* lipase were investigated systematically. The best reaction medium was found to be acetone or acetonitrile. The optimal enzyme dosage, molar ratio of vinyl caproate to floxuridine, and reaction temperature were 40 U, 7.5, and 35 °C, respectively. Under the optimal conditions, a substrate conversion of >99% and a regioselectivity of 93.5% could be achieved after a reaction time of 4 h. The half life of *P. cepacia* lipase was less than 4 batches with acetone or acetonitrile as the solvent.
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Keywords: *Pseudomonas cepacia* lipase; Floxuridine; 3′-*O*-Caproyl-floxuridine; Regioselective acylation; Vinyl caproate

1. Introduction

Floxuridine(5-fluoro-2′-deoxyuridine, FUdR) is a fluorinated deoxynucleoside analogue, which has been used extensively in the clinical treatment of colon carcinoma and hepatic metastases for over 40 years [1]. The mechanisms of action of FUdR have been well understood [2]. Namely, FUdR is phosphorylated intracellularly to yield 5′-phosphate of FUdR, the specific competitive inhibitor for thymidylate synthetase, resulting in the inhibition of DNA biosynthesis. FUdR has proven to be active in the treatment of various cancers. However, it exhibits various side effects due to its actions on the gastrointestinal tract and bone marrow [3]. Moreover, FUdR, like other nucleoside antitumor analogs, suffers from low and erratic oral bioavailability due to its poor cell membrane penetrability. Hence, FUdR is customarily intravenously administered [4]. At the same time, FUdR would be degraded to 5-fluorouracil (5-FU) as a result of glycosidic bond metabolism. The anticancer activity of 5-FU is 5000-fold lower than that of FUdR. It is well understood that it is thymidine nucleoside phosphorylase that could catalyze the cleavage of glycosidic bond [5]. Therefore, chemical modification is aimed at increasing the intestinal uptake of FUdR, thus meliorating its oral bioavailability. In addition, the modification should also address the issue of how to prevent the transformation of FUdR to its less active metabolite, 5-FU [6–9]. Chemical modification of parent compounds is one of the most successful strategies to overcome the drawbacks as described above. For example, lipophilic nucleoside prodrugs that could cross the cell membrane and liberate parent agents have been widely investigated. Nishizawa et al. [8] prepared a group of C4 to C18 aliphatic acid esters of FUdR, and found that *O*-butanoyl esters were more effective than parent agent following oral administration to mice with adenocarcinoma-775 tumors. Retinoate of FUdR, a dual prodrug, not only exhibited comparable cytotoxity with FUdR against 60 human tumor cell lines, but also induced *in vitro* cell differentiation of promyelocytic leukemia HL60 cells and *in vivo* delayed tumor growth [7].

There are two hydroxyl groups with similar chemical reactivity in the FUdR molecule. It is usually extremely difficult to selectively acylate the desired hydroxyl of unprotected FUdR directly via the conventional organic synthesis approaches. Generally, time-consuming protection–unprotection steps are

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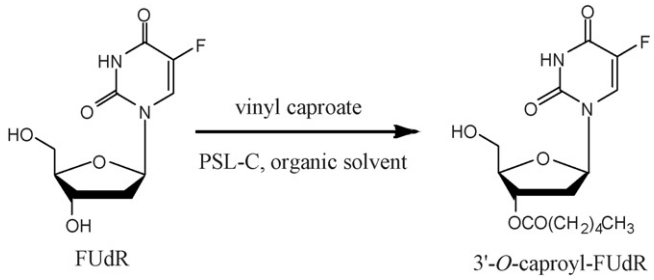

Scheme 1. The synthesis of 3′-*O*-caproyl-FUdR catalyzed by PSL-C.

needed [10]. Doubtlessly, regioselective acylation of FUdR mediated by enzymes is an artful approach. Enzymatic acylation of nucleosides and related compounds has been well established [11,12]. Indeed, Nozaki and co-workers [13] reported the enzymatic regioselective acylation of the 3′-hydroxyl of FUdR and 2′-deoxy-5-trifluoromethyluridine. In dioxane, the reaction underwent in a good efficiency for FUdR and related substrates. For example, 3′-acetate was obtained in 80% yield. In this paper we describe the intensive elaboration for the conditions, especially on various reaction parameters, based on our previous examination [14–16]. Highly regioselective caproylation of FUdR catalyzed by an immobilized lipase from *Pseudomonas cepacia* (PSL-C) was realized under mild conditions (Scheme 1).

2. Materials and methods

2.1. Materials

FUdR was purchased from Shanghai Hanhong Co., Ltd., China. Vinyl caproate was bought from TCI, Japan. Novozym 435 (an immobilized lipase from *Candida antarctica*), Lipozyme TL IM (an immobilized lipase from *Thermomyces lanuginosus*), Lipozyme RM IM (an immobilized lipase from *Rhizomucor miehei*), and Lipozyme IM (an immobilized lipase from *Mucor miehei*) were purchased from Novozymes Co., Ltd., China. PSL-C (an immobilized lipase from *P. cepacia*) was from Amamo Enzyme Inc., Japan. Lipase MY (powder, lipase from *Candida cylindracea*) and lipase OF (powder, lipase from *C. cylindracea*) were obtained from Meito Sangyo Co., Ltd., Japan. All other chemicals are of high purity commercially available.

2.2. Determination of FUdR solubility in organic solvents

The solubility of FUdR in organic solvents was determined by HPLC analysis of the saturated solutions at 25 °C.

2.3. Assaying of enzyme esterification activity [17]

Certain amount of enzyme was added to a screw capped vial containing a mixture of lauric acid (1 mmol), *n*-propanol (2 mmol) and 3 ml water-saturated isooctane. The vials were placed in a shaker at 37 °C, 250 rpm. After a specified time, the reaction was stopped by addition of 10 ml ethanol, and the mixture was immediately titrated for the unreacted fatty acid against 0.05 M alcoholic NaOH solution using phenolphthalein as indicator. One unit is the amount of the enzyme which catalyzes the formation of 1 μmol propyl laurate in 1 min at 37 °C. The specific esterification activities of Novozym 435, Lipozyme TL IM, Lipozyme RM IM, Lipozyme IM, PSL-C, lipase MY and lipase OF are 2875, 433, 960, 2525, 730, 412 and 415 U/g, respectively.

2.4. Control of the initial water activity

The solvents were dried by gentle shaking with 4 Å molecular sieves overnight. The initial water activities (a_w) of the anhydrous solvents, the substrates and the enzymes were controlled by gaseous equilibrium with different saturated salt solutions in separate closed containers for 72 h at 25 °C. The following salts were used: LiBr ($a_w = 0.07$), LiCl ($a_w = 0.11$), CH_3COOK ($a_w = 0.23$), $MgCl_2$ ($a_w = 0.33$), K_2CO_3 ($a_w = 0.43$), $Mg(NO_3)_2$ ($a_w = 0.53$), NaCl ($a_w = 0.75$), K_2SO_4 ($a_w = 0.97$) [15,18].

2.5. General procedure for enzymatic acylation of FUdR

In a typical experiment, 2 ml of organic solvent containing FUdR (20 mM), vinyl caproate (100 mM) and enzyme was incubated in a 10 ml Erlenmeyer shaking flask capped with a septum at 250 rpm and 35 °C. Aliquots were withdrawn at specified time intervals from the reaction mixture, and then diluted by 25 times with corresponding mobile phase prior to HPLC analysis.

2.6. 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 267 nm and 1 ml/min. A gradient elution with water/methanol being 60/40 (v/v) from 0 to 2.8 min, and then water/methanol being 5/95 (v/v) at 6.0 min was used. The retention times for FUdR, 5′-, 3′-caproate and 3′,5′-dicaproate of FUdR were 2.54, 8.40, 8.63, and 9.71 min, respectively. Regioselectivity was defined as the ratio of the HPLC peak area of the indicated product to those of all the products [16]. The initial reaction rate (V_0) and the substrate conversion (conv.) were calculated from the HPLC data.

2.7. Purification and structure determination of the esters

Upon the completion of the reaction, the enzyme was filtered off, and the filtrate was concentrated under vacuum. The residue was separated and purified through flash column chromatography using petroleum ether/ethyl acetate (11/5, v/v) as the mobile phase. The structures of FUdR ester derivatives (Table 1, ^{13}C NMR) were determined by ^{13}C NMR and ^{1}H NMR (Bruker DRX 400 MHz NMR spectrometer, Germany) at 100.5 and 400 MHz, respectively. Acetone-d_6 was used as solvent.

3′-*O*-Caproyl-FUdR: ^{1}H NMR (acetone-d_6) δ: 0.94 (t, 3H, $C_{6''}$); 1.34–1.39 (m, 4H, $C_{4''}$, $C_{5''}$); 1.67 (t, 2H, $C_{3''}$); 2.40–2.45 (m, 4H, $C_{2''}$, $C_{2'}$); 3.93 (m, 2H, $C_{5'}$); 4.16 (d, 1H, $C_{4'}$); 4.62 (t, 1H, OH); 5.41 (m, 1H, $C_{3'}$); 6.35 (m, 1H, $C_{1'}$); 8.30 (d, 1H, C_6); 10.51 (s, 1H, NH).

Table 1
^{13}C NMR spectral data for FUdR and its ester derivatives (δ, ppm)

Carbon no.	FUdR	3′-*O*-Caproyl-FUdR	5′-*O*-Caproyl-FUdR	3′,5′-*O*-Dicaproyl-FUdR
Base ring				
C_2	150.24	150.27	150.13	150.16
C_4	158.01	157.92	157.94	157.86
C_5	141.65	141.82	141.75	141.85
C_6	125.84	125.50	125.25	125.02
Sugar moiety				
$C_{1'}$	86.46	86.82	86.42	86.45
$C_{2'}$	41.73	38.80	40.99	38.06
$C_{3'}$	72.34	76.24	72.00	75.23
$C_{4'}$	89.15	86.35	85.88	83.59
$C_{5'}$	62.94	63.24	64.68	64.74
Acyl moiety				
$C_{1''}$		173.84	173.89	173.78
$C_{2''}$		34.86	34.77	34.74
$C_{3''}$		32.27	32.27	32.24
$C_{4''}$		25.55	25.62	25.49
$C_{5''}$		23.33	23.29	23.30
$C_{6''}$		14.54	14.51	14.53

5′-*O*-Caproyl-FUdR: ^{1}H NMR (acetone-d_6) δ: 0.92 (t, 3H, $C_{6''}$); 1.34–1.37 (m, 4H, $C_{4''}$, $C_{5''}$); 1.67 (m, 2H, $C_{3''}$); 2.35–2.45 (m, 4H, $C_{2''}$, $C_{2'}$); 4.16 (m, 1H, $C_{3'}$); 4.31–4.44 (m, 2H, $C_{5'}$); 4.51 (t, 1H, $C_{4'}$); 4.69 (s, 1H, OH); 6.32 (m, 1H, $C_{1'}$); 7.91 (d, 1H, C_6); 10.55 (s, 1H, NH).

3′,5′-*O*-Dicaproyl-FUdR: ^{1}H NMR (acetone-d_6) δ: 1.39 (t, 6H, $C_{6''}$); 1.81 (m, 8H, $C_{4''}$, $C_{5''}$); 2.12 (m, 4H, $C_{3''}$); 2.87–2.98 (m, 6H, $C_{2''}$, $C_{2'}$); 4.78–4.83 (m, 2H, $C_{5'}$); 4.93 (m, 1H, $C_{4'}$); 5.80 (d, 1H, $C_{3'}$); 6.76 (t, 1H, $C_{1'}$); 8.37 (d, 1H, C_6); 11.04 (s, 1H, NH).

3. Results and discussion

3.1. Regioselectivity and substrate conversion of the acylation of FUdR with various enzymes

Lavandera et al. [19,20] reported that the lipase could functionalize its favorable hydroxyl in the acylation of polyhydroxyl compounds, due to the specific structure of its active center. Also, when vinyl ester acted as the active acyl donor, the released by-product of this reaction, acetaldehyde, might cause a dramatic loss in the activity possibly because of the Schiff's base formation of lysine residues in the lipase structure. It was previously reported by Weber et al. [21] that the lipases from *R. miehei, C. antarctica* B and *Pseudomonas glumae* were shown to be remarkably stable, while the lipases from *Candida rugosa* and *Geotrichum candidum* lost most of their activity when exposed to acetaldehyde. The difference in the lipases' sensitivity towards acetaldehyde proved to be closely related to their molecular structure. Therefore, several commercially available microbial lipases were initially tested due to their potential in catalyzing the regioselective acylation of FUdR with vinyl caproate (Table 2). The results showed that 5′-caproate was the favorable regiomer for Novozym 435 (*C. antarctica* B lipase), Lipozyme TL IM (*T. lanuginosus* lipase), Lipozyme RM IM (*R. miehei* lipase), and Lipozyme IM (*M. miehei* lipase), while the favorable formation of 3′-caproate was found on other enzymes. According to Gotor and co-workers [20], the reason of the preferential acylation of 5′-hydroxyl of FUdR mediated by Novozym 435 is that the base ring is situated on the right side of the large hydrophobic pocket in this enzyme, making it the most productive conformation due to better enzyme–substrate interactions. The molecular basis of high 3′-regioselectivity of

Table 2
Acylation of FUdR with vinyl caproate catalyzed by various enzymes

	V_0 (mM/h)	Conversion (%)	Regioselectivity (%)		
			5′-Caproate	3′-Caproate	3′,5′-Dicaproate
Novozym 435	15.1	95.4	78.2	15.1	6.7
Lipozyme TL IM	17.9	67.7	54.4	38.2	7.4
Lipozyme RM IM	0.6	21.0	81.6	18.4	0.0
Lipozyme IM	0.9	37.3	78.6	16.6	4.8
PSL-C	27.2	99.4	2.3	93.9	3.8
Lipase MY	1.5	20.9	25.0	75.0	0.0
Lipase OF	2.2	22.0	26.1	73.9	0.0

The reaction was initiated by adding 50 U enzyme into 2 ml of anhydrous acetone containing FUdR (20 mM) and vinyl caproate (100 mM) and then incubated at 35 °C, 250 rpm.

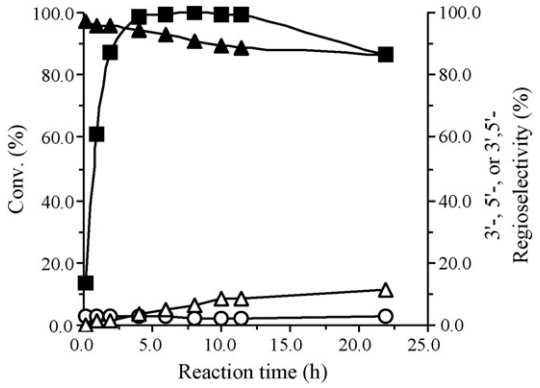

Fig. 1. Progress curve of PSL-C-catalyzed acylation of FUdR. The reaction was initiated by adding 40 U PSL-C into 2 ml of anhydrous acetone containing FUdR (20 mM) and vinyl caproate (100 mM) and carried out at 35 °C, 250 rpm. Symbols: (■) conversion; (▲) 3′-regioselectivity; (◯) 5′-regioselectivity; (△) 3′,5′-regioselectivity.

PSL-C was also proposed by Gotor and co-workers [19]. It is the remote stabilizing interactions, including hydrogen bonds and hydrophobic interactions, between the FUdR base and PSL-C substrate-binding site that stabilize the 3′-acylation transition state and thus result in the high regioselectivity. As showed in Table 2, both Novozym 435 and PSL-C exhibited high activity, while the reaction catalyzed by lipase MY and OF (both from *C. rugosa*) proceeded with low reaction rate and low conversion, partially owing to the difference of the lipases' sensitivity towards acetaldehyde.

3.2. Time course of enzymatic acylation of FUdR

In order to follow the enzymatic reaction process, the time course of PSL-C-mediated acylation of FUdR with vinyl caproate was studied (Fig. 1). As shown in Fig. 1, the conversion went up dramatically with reaction time, and reached its maximum at a reaction time of 4 h. During this periods, 3′-caproate was the dominant products with regioselectivity of >94%. The conversion remained almost unchanged for the following 7.5 h, and then it began to drop slowly. The selectivity towards 5′-caproate remained the same during the whole reaction process, whereas that of 3′,5′-dicaproate increased with the elongation of time. It is interesting to note that the decrease of 3′-regioselectivity matches the increase of selectivity towards 3′,5′-dicaproate. Obviously, part of 3′-caproate was further esterified to dicaproate with the progress of the reaction. The gradual drop of substrate conversion after 11.5 h reaction was possibly due to the hydrolysis of 3′-*O*-caproyl-FUdR. Hence, it is of significant importance to control reaction time in order to obtain high conversion and high 3′-regioselectivity in this enzymatic reaction.

3.3. Effect of enzyme dosage

As shown in Fig. 2, the initial reaction rate underwent a dramatic increase when the enzyme dosage changed from 10 to 40 U, and then kept constant at around 25.3 mM/h with the enzyme dosage more than 40 U. In addition,

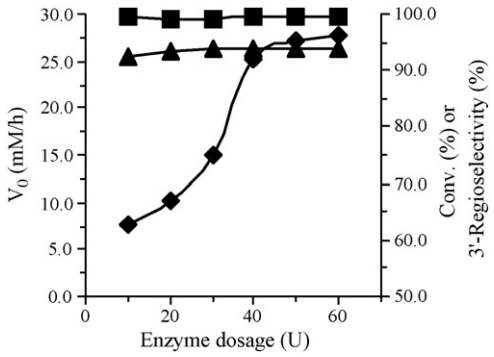

Fig. 2. Effect of enzyme dosage on PSL-C-catalyzed acylation of FUdR. The reactions were carried out at 35 °C, 250 rpm by adding various dosages of PSL-C into 2 ml of anhydrous acetone containing FUdR (20 mM) and vinyl caproate (100 mM). Symbols: (♦) V_0; (■) conversion; (▲) 3′-regioselectivity.

enzyme dosage showed a slight effect on the conversion and 3′-regioselectivty.

3.4. Effect of initial water activity

Water activity plays a crucial role in nonaqueous enzymology [16,22]. Water, acting as a lubricant or plasticizer, allows enzymes to have the conformational mobility to construct optimal active center for a specific catalytic reaction. On the other hand, the presence of water may result in the competitive hydrolysis of desired products and acyl donor in the enzymatic transesterification. Hence, the optimal water amount represented the most appropriate water condition from the trade-off of the conflicts mentioned above. From Table 3, PSL-C-catalyzed acylation of FUdR showed a clear a_w dependence in acetone. The largest conversion was obtained when the enzyme was used as-received and the solvent acetone was anhydrous, which suggested that the moisture content of the enzymatic preparation was enough for enzyme to exhibit its catalytic activity. With the increase of a_w, the reaction equilibrium was reached in a short time, and the maximal conversion decreased drastically. In addition, only trace of ester derivatives was observed at $a_w > 0.11$, possibly owing to the hydrolysis of acyl donor in the present of excessive water. Also, the enzymatic degradation of the nucleoside esters at higher a_w could account for this. There-

Table 3
Effect of initial water activity on PSL-C-catalyzed acylation of FUdR

a_w	Time (h)	Conversion (%)	3′-Regioselectivity (%)
0[a]	4	98.5	93.5
0.07	3	12.8	95.7
0.11	2	7.0	>99.9
0.22	2	6.4	>99.9
0.33	2	6.2	>99.9
0.43	2	4.1	>99.9
0.55	2	2.2	>99.9
0.75	2	2.1	>99.9
0.97	19.5	N.D.	N.D.

[a] Enzyme used as-received, anhydrous acetone. The reactions were carried out at 35 °C, 250 rpm by adding 40 U PSL-C, FUdR (20 mM) and vinyl caproate (100 mM) into 2 ml of acetone with controlled water activity.

Table 4
Effect of reaction medium on the PSL-C-catalyzed acylation of FUdR

Medium	log *P*	Solubility (mM)	V_0 (mM/h)	Conversion (%)	3′-Regioselectivity (%)
Acetonitrile	−0.33	40.4	28.7	99.4	91.1
Acetone	−0.23	81.9	25.0	>99.9	93.4
t-Butanol	0.6	31.9	8.7	93.5	83.3
Dioxane	−1.1	140.1	7.5	35.6	98.7
THF	0.49	154.9	3.7	19.8	97.9
Pyridine	0.71	>1172.6	6.4	55.0	95.3
DMSO	−1.3	>1447.8	N.D.	N.D.	N.D.
DMF	−1.0	>2062.8	N.D.	N.D.	N.D.

The reactions were carried out at 35 °C, 250 rpm by adding 40 U PSL-C, FUdR (20 mM) and vinyl caproate (100 mM) into 2 ml of anhydrous solvent.

fore, the standard commercial preparation of the catalyst was used for the subsequent investigations with no water activity control.

3.5. Effect of reaction medium

One of the obstacles in enzymatic acylation of nucleosides lies in their poor solubility in hydrophobic solvent, a friendly medium for the enzyme. In most of the previous reports, hydrophilic solvents, such as pyridine, THF and DMF, were used as the reaction media in the enzymatic modification of nucleosides [15,23]. However, hydrophilic solvents are prone to destroy the essential water shell on the surface of enzyme, and therefore deactivate enzyme, which limits the application of enzymatic approaches in this field [22]. On the other hand, the selectivity, such as regioselectivity [24], enantioselectivity [25] and substrate selectivity [26] could be manipulated by the reaction medium.

As shown in Table 4, although the solubility of FUdR was high in the highly hydrophilic solvents (DMSO and DMF), no ester derivatives were observed. *t*-Butanol, a nontoxic solvent, was usually used as the medium for the enzymatic acylation of polyhydroxyl compounds, such as disaccharides [27]. And it is not a substrate for lipases because it is too sterically hindered to enter the enzymes' active site. However, *t*-butanol is a liquid with high viscosity, which might cause mass transfer limitation. Possibly for the reason, low reaction rate was observed and more time was needed to reach a high conversion with *t*-butanol as a solvent, which resulted in a lower regioselectivity. The solubility of FUdR was moderate in acetone and acetonitrile. However, the reaction proceeded with much higher reaction rate, substrate conversion and 3′-regioselectivity in the two solvents. Although 3′-acetate of FUdR was prepared by Nozaki et al. [13] with satisfied yield in dioxane, the conversion of 3′-caproate of FUdR in this medium was low, as showed in Table 4. Additionally, dioxane as the medium is more toxic than acetone and acetonitrile. While THF was one of commonly used solvents in the acylation of natural nucleosides [23], it was not a suitable reaction medium for this reaction as indicated by the low reaction rate and poor conversion under the conditions tested. Also, the results showed that the catalytic activity of the lipase was not well correlated with log *P* of the medium, the most widely used solvent parameter in the field of nonaqueous enzymology.

3.6. Effect of molar ratio of vinyl caproate to FUdR

In enzymatic acylation of nucleosides with vinyl esters, there exits a side reaction that acyl donors could be hydrolyzed by the enzyme. Hence, excessive amount of acyl donors is normally necessary for enzymatic acylation of nucleosides [16]. When FUdR concentration was fixed at 20 mM, the enzymatic caproylation of FUdR was greatly affected by the molar ratio of vinyl caproate to FUdR (Fig. 3). As one can see in Fig. 3, a lower molar ratio resulted in a lower initial reaction rate and a lower conversion, because the hydrolysis of vinyl caproate was unfavorable to push the equilibrium toward the synthesis of the desired esters. Remarkable enhancement in both initial reaction rate and maximal conversion was observed with the increase of molar ratio up to 7.5, which was the optimal ratio of vinyl caproate to FUdR. On the contrary, the influence of the molar ratio on the regioselectivity was negligible, which was consistent with our recent report [16].

3.7. Effect of reaction temperature

As we know, the higher the reaction temperature, the more active the substrate molecules. The increase of temperature leads to a higher reaction rate apparently. On the other hand, enzymes are biomacromolecules, which are apt to defold at high temperatures. The influence of temperature on the reaction rate, substrate

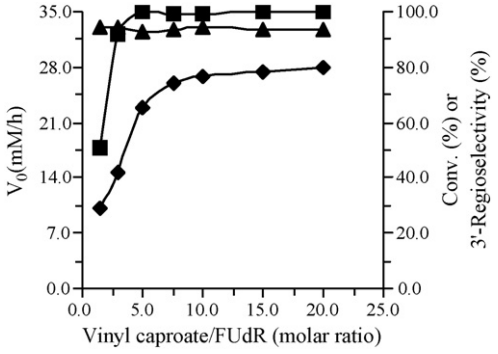

Fig. 3. Effect of the molar ratio of vinyl caproate to FUdR on the enzymatic reaction. The reactions were carried out at 35 °C, 250 rpm by adding 40 U PSL-C into 2 ml of anhydrous acetone containing FUdR (20 mM) and corresponding concentration of vinyl caproate. Symbols: (♦) V_0; (■) conversion; (▲) 3′-regioselectivity.

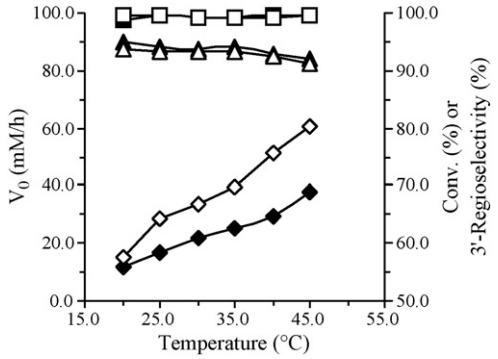

Fig. 4. Effect of reaction temperature on the enzymatic reaction. The reactions were carried out at different temperatures, 250 rpm by adding 40 U PSL-C into 2 ml of anhydrous acetone (solid) or acetonitrile (hollow) containing FUdR (20 mM) and vinyl caproate (150 mM). Symbols: (♦) V_0; (■) conversion; (▲) 3′-regioselectivity.

conversion and 3′-regioselectivity was investigated (Fig. 4). The results showed that the initial reaction rate increased rapidly with the increase of the reaction temperature in both solvents within the range from 20 to 45 °C. However, higher temperature caused a slight drop of selectivity to 3′-position. More 3′,5′-dicaproate formation at higher temperature could be responsible for this, which was confirmed by HPLC analysis.

3.8. Operational stability

In this reaction system, acetone or acetonitrile, a hydrophilic organic solvent, acted as the reaction medium, which was apt to strip the essential water off the enzyme molecules and thereby deactivate the enzyme [22]. On the other hand, acetaldehyde, the side product of the reaction, would couple with ε-amino groups of lysine at the active site, which also resulted in deactivation of enzyme [21]. The comparison of operational stability of PSL-C-catalyzed acylation was carried out in acetone, acetonitrile and dioxane (Fig. 5). As could been seen in Fig. 5, the operational stability of PSL-C was comparable in acetone and acetonitrile, and the half life of PSL-C was less than 4 batches (5 h per batch)

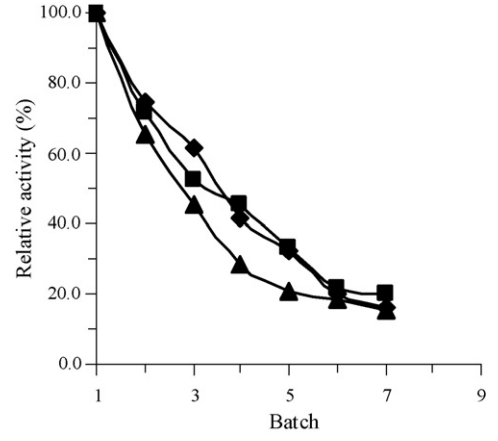

Fig. 5. The operational stability of PSL-C in organic media. The reactions were carried out at 35 °C, 250 rpm by adding 40 U PSL-C into 2 ml of anhydrous acetone (♦), acetonitrile (■) or dioxane (▲) containing FUdR (20 mM) and vinyl caproate (150 mM).

in both solvents. Moreover, the stability of PSL-C was poorer in dioxane than that in acetone and acetonitrile. In terms of the operational stability, unfortunately, the three solvents were not the appropriate reaction media. Based on previous reports, we propose that the operational stability might be enhanced significantly in a co-solvent mixtures by adding a hydrophobic solvent [15] or ionic liquid [14] into acetone or acetonitrile, which is still under investigation. Additionally, oxime ester [28] could be used as the acyl donor, which would avoid deactivating the enzyme by the side product acetaldehyde.

4. Conclusions

In conclusion, we have successfully synthesized 3′-*O*-caproyl-FUdR by selective acylation of the parent compound mediated by PSL-C with vinyl caproate. Under the optimal conditions, a substrate conversion of >99% and a regioselectivity of 93.5% were achieved after a reaction time of 4 h. The use of acetone or acetonitrile as a solvent avoided the tedious work-up of the reaction with other solvents traditionally used in nucleoside chemistry such as pyridine or DMSO. However, both solvents were not good enough in terms of their operational stability. Future work about the co-solvent system is in process.

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