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Regioselective acylation of nucleosides and their analogs catalyzed by *Pseudomonas cepacia* lipase: enzyme substrate recognition

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ABSTRACT

The substrate recognition of *Pseudomonas cepacia* lipase in the acylation of nucleosides was investigated by means of rational substrate engineering for the first time. *P. cepacia* lipase displayed excellent 3′-regioselectivities (96 to >99%) in the lauroylation of 2′-deoxynucleosides **1a**–**1e**, while low to good 3′-regioselectivities (59–89%) in the lauroylation of ribonucleosides **1f**–**1j**. It might be due to the unfavorable hydrogen bond interaction between 2′-hydroxyl group of **1f**–**1j** and phenolic hydroxyl group of tyrosine residue present in the alternate hydrophobic pocket of the enzyme, which stabilizes the conformation of 5′-acylation transition state and thus increases the amount of the minor regioisomer. In addition, various ester derivatives of floxuridine were synthesized successfully by the lipase with high conversions (99%) and good to excellent 3′-regioselectivities under mild conditions. The recognition of various acyl donors by the enzyme was examined. The enzymatic recognition of acyl groups was rationalized in terms of the structure of the active site of the lipase, especially the size, shape, and physicochemical properties.

1. Introduction

The regioselective acylation of nucleosides remains a tedious, yet important, task in organic synthesis due to the presence of multiple hydroxyl groups. In particular, it is difficult to selectively acylate the 3′-hydroxyl of nucleosides in the presence of less hindered 5′-hydroxyl, since the former is typically less reactive than the latter. For example, acyl groups such as pivaloyl and aroyl favored the primary 5′-hydroxyl of nucleosides.[1] Hence, the conventional acylation of the 3′-hydroxyl involves protecting/deprotecting steps, which is a time-consuming work-up.[2] In the past 20 years, enzymatic acylation of polyhydroxyl compounds has attracted increasing synthetic attention, due to the exquisite selectivity, mild reaction conditions, and being environmentally friendly.[3] Among the enzymes, *P. cepacia* lipase (PSL-C) has proven to be highly selective toward 3′-position in the acylation of nucleosides.[4] Recently, Lavandera et al. revealed the molecular basis of the unusual regioselectivity of PSL-C toward the secondary hydroxyl of 2′-deoxynucleosides by molecular modeling.[5]

Nucleoside analogs, such as floxuridine (FUdR, **1b**) and idoxuridine (**1e**) have been acting as effective antitumor or antiviral agents for many years.[6] However, these halogenated nucleoside analogs usually exhibit various side effects and suffer from poor bioavailability in the clinical treatment.[7] Moreover, nucleoside agents are less stable in vivo because of the cleavage of the glycosyl bond by nucleoside phosphorylase, yielding the compounds of lower biological activity.[8] It has been demonstrated that ester derivatives of parent drugs could effectively circumvent the drawbacks mentioned above.[2b,9] For example, 3′-*O*-retinoyl-FUdR could remarkably inhibit in vivo solid tumor growth as compared to the parent drug.[10] 3′-Acetate and amino acid ester derivatives of FUdR are much more resistant to nucleoside phosphorylase than FUdR.[8,11]

Previously, enzymatic regioselective approaches for the acylation of nucleoside analogs, such as 1-β-D-arabinofuranosylcytosine,[12] 5-fluorouridine,[13] FUdR and its analogs,[14] were developed by our group. In the present work, we continued to extend our interest to the enzymatic synthesis of various lipophilic 3′-ester derivatives of nucleosides and their analogs in high conversions and excellent regioselectivities. Particularly, we focused on the substrate recognition of PSL-C in the acylation of nucleosides by means of rational substrate engineering (Scheme 1).

2. Results and discussion

Recently, we found that the regioselectivity and reaction rate of the enzymatic benzoylation of FUdR **1b** could be enhanced significantly by adding ionic liquid.[14a] Likewise, herein, the ionic

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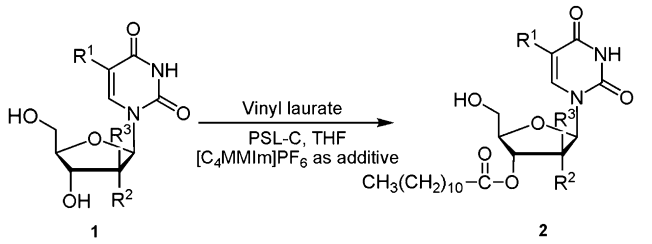


	R^1:	R^2:	R^3:		R^1:	R^2:	R^3:
a	H	H	H	**f**	H	OH	H
b	F	H	H	**g**	F	OH	H
c	CH_3	H	H	**h**	CH_3	OH	H
d	Br	H	H	**i**	Br	OH	H
e	I	H	H	**j**	I	OH	H
k	H	F	H	**m**	H	H	OH

Scheme 1. PSL-C-mediated 3′-lauroylation of nucleosides.

liquid-containing system was used for the enzymatic acylation of nucleosides. Upon optimization, $[C_4MMIm]PF_6$ (5 mg/mL) turned out to be the optimal additive (unpublished data).[15] The effect of nucleoside structure, especially R^1, R^2, and R^3 groups, on the enzymatic reaction was examined by using vinyl laurate as the acyl donor (Table 1). It was found that 2′-deoxynucleosides **1a**–**1e** could be transformed to the desired 3′-ester derivatives with high conversions (99%) and excellent regioselectivities (96 to >99%) by PSL-C in 3–6 h. Furthermore, the 3′-regioselectivity increased with increasing hydrophobicity of R^1 group of 2′-deoxynucleoside, which was in agreement with our previous report.[14a] It might be due to the favorable remote interaction between R^1 group and the hydrophobic side chain of amino acid residue Leu287 present in the active site of PSL-C in the conformation of 3′-acylation transition state.[5] A more hydrophobic R^1 group would result in a stronger interaction, stabilizing this conformation and thus increasing the amount of the major regioisomer. In addition, the reaction rate decreased with increasing bulk of R^1 group, possibly due to the steric strain. For example, 2′-deoxyuridine **1a** (R^1=H) disappeared in the mixture after the reaction time of 3 h, whereas β-thymidine **1c** (R^1=CH_3) and idoxuridine **1e** (R^1=I) were completely acylated to the ester derivatives after 3.5 and 6 h, respectively.

Table 1
Effect of substrate structure on PSL-C-mediated lauroylation of nucleosides[a]

Nucleoside	Time (h)	Conversion[b] (%)	3′-Regioselectivity[c] (%)
1a	3	>99	96
1b	4	>99	99
1c	3.5	>99	>99
1d	5	>99	>99
1e	6	99	>99
1f	14	99	59
1g	22	99	78
1h	16	98	89
1i	30	98	86
1j	35	98	88
1k	12	98	92
1m	30	99	69

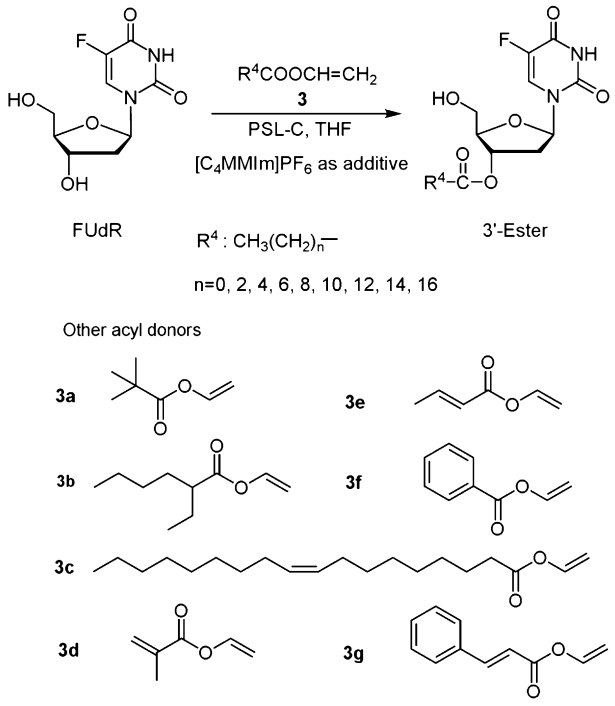
[a] The reaction was carried out at 40 °C, 250 rpm by adding 40 U PSL-C into anhydrous THF (2 mL) containing nucleosides (0.04 mmol), vinyl laurate (0.16 mmol) with $[C_4MMIm]PF_6$ (10 mg) as the additive.
[b] Determined by HPLC analysis using SB-C18 column.
[c] Defined as the ratio of the concentration of the desired product to that of all the products, and determined by HPLC analysis using SB-C18 column.

As can be seen in Table 1, 3′-laurates of ribonucleosides **1f**–**1j** were synthesized with high conversions (>98%) and low to good selectivities (59–89%) in 14–35 h. Interestingly, PSL-C exhibited similar catalytic behavior in the acylation of the two series of nucleosides (2′-deoxynucleosides **1a**–**1e** and ribonucleosides **1f**–**1j**). For instance, a poor 3′-regioselectivity (59%) and the highest catalytic activity were observed in the acylation of uridine **1f** (corresponding to 2′-deoxyuridine **1a**) among **1f**–**1j**, while a good 3′-regioselectivity (88%) and the lowest activity were found in the acylation of 5-iodouridine **1j** (corresponding to idoxuridine **1e**). It was suggested that the orientation and binding of the substrates in the active site of PSL-C might be similar for the acylation of both 2′-deoxynucleosides **1a**–**1e** and ribonucleosides **1f**–**1j**. However, lower 3′-regioselectivities and slower reaction rates were observed in the acylation of ribonucleosides **1f**–**1j** as compared to those in the acylation of 2′-deoxynucleosides **1a**–**1e**, due to the presence of 2′-hydroxyl group. Lavandera et al. proposed the model of phosphonate analogs for PSL-C-mediated butanoylation of β-thymidine **1c** by molecular modeling.[5] According to the model, the C2′ of sugar moiety is located above the medium-sized pocket of PSL-C and points into the solvent in the conformation of 3′-acylation transition state, while the C2′ points into the alternate hydrophobic pocket of the enzyme in 5′-acylation. It has been proven that there exist several tyrosine residues such as Tyr23 and Tyr29 in the alternate hydrophobic pocket.[16] As we know, the hydroxyl group is a good acceptor or donor of hydrogen bond. The lower 3′-regioselectivities in the acylation of ribonucleosides **1f**–**1j** might be a result of the unfavorable hydrogen bond between 2′-hydroxyl and the phenolic hydroxyl of the tyrosine residue such as Tyr29 of the enzyme, which stabilizes the conformation of 5′-acylation transition state, and thus increases the amount of the minor regioisomer. Kazlauskas et al. proposed that the enantioselectivity derived from an extra hydrogen bond between the phenoxy oxygen of the substrate to the phenolic hydroxyl of Tyr29 of the enzyme in PSL-C-catalyzed resolution of 2-phenoxy-1-propanol.[16] The lower catalytic activity of PSL-C might partially be attributed to the steric hindrance of 2′-hydroxyl group in the synthesis of the ester derivatives of ribonucleosides **1f**–**1j**.

The hypothesis was confirmed by the results from the acylation of 2′-fluoro-2′-deoxyuridine **1k** and 1-β-D-arabinofuranosyluracil **1m** catalyzed by PSL-C (Table 1). Like **1a**, a good 3′-regioselectivity (92%) was achieved in the acylation of **1k**, since it is difficult to make a hydrogen bonding between 2′-F and the phenolic hydroxyl of tyrosine. Besides, 3′-laurate of **1m** was synthesized with the selectivity of 69%, which is slightly higher than that (59%) in the acylation of **1f**. The conformation of 2′-hydroxyl group of **1m** is different from that of **1f**. Perhaps the donor atom (2′-OH of **1m**) is too far from the acceptor atom (phenolic OH of tyrosine residue) to form a hydrogen bond. As a result, a weaker close interaction rather than a hydrogen bond interaction occurred between 2′-hydroxyl of **1m** and phenolic hydroxyl of tyrosine residue, resulting in a higher 3′-regioselectivity. Previously, a moderate 3′-regioselectivity (80%) was reported in PSL-C-mediated levulinylation of 2′-*O*-alkyl-5-methyluridine,[4a] being similar to that (89%) in the lauroylation of 5-methyluridine **1h**. It implies that the oxygen of 2′-hydroxyl group of **1f**–**1j** might act as an acceptor of hydrogen bond, while phenolic hydroxyl of tyrosine as a donor.

Then, FUdR was used as a model acyl acceptor and its regioselective acylation catalyzed by PSL-C was attempted with vinyl esters as the acyl donors (Scheme 2). Initially, the chain length specificity (C2–C18) of the lipase was examined (Fig. 1). As shown in Figure 1, PSL-C exhibited the highest acylation activity (48.2 mM/h) toward vinyl acetate, and the reaction rate decreased with the elongation of the chain. It is well known that *P. cepacia* lipase has a funnel-like binding site.[17] Therefore, a longer acyl donor might be

more difficult to enter into the active site to form the first tetrahedron intermediate (generally considered as the rate-limiting step), due to the steric strain. Similar phenomenon was observed in the regioselective acylation of 5-fluorouridine **1g** mediated by PSL-C.[18] Interestingly, the 3′-regioselectivity increased with the lengthening chain from C2 to C8, and PSL-C displayed excellent 3′-regioselectivities (>99%) in the enzymatic acylation of FUdR with fatty acid vinyl esters of more than C8 as acyl donors. According to the model proposed by Lavandera et al.,[5] the increasing 3′-regioselectivity might be due to the destabilization of the conformation of 5′-acylation transition state by a larger acyl group. The R^1 group of base moiety extends into the large hydrophobic pocket, into which the acyl group also binds. A larger acyl group might result in steric clash, and destabilize the conformation of 5′-acylation transition state, thus leading to the decrease of the amount of the minor regioisomer.



Scheme 2. Regioselective acylation of FUdR catalyzed by PSL-C.

Finally, the effect of the substituent or functional group present in the acyl donor on the enzymatic acylation of FUdR was investigated (Table 2). It was shown that the reaction rate dropped remarkably with branched fatty acid vinyl esters as acyl donors (Table 2, entries 1–4), perhaps due to the steric hindrance. For example, a conversion of 67% was achieved for the pivaloylation after 47 h (entry 1), while FUdR was butanoylated with a conversion of >99% after 2 h. In addition, the larger the substituent is, the slower the reaction rate becomes. With vinyl 2-ethylhexanoate as the acyl donor, a low conversion (10%) was obtained after 47 h (entry 3). Increasing the reaction temperature and molar ratio of acyl donor to FUdR could afford improved results (entries 2 and 4). It is worth noting that no 3′,5′-diesters were detected in the pivaloylation and 2′-ethylhexanoylation even at high temperature (50 °C). It could be attributed to the steric hindrance created by the acyl groups of the monoesters. For the same reason, Gotor et al.'s effort to synthesize the dibenzoate from 5′-*O*-protected nucleoside by PSL-C failed.[19] When C–C double bond in the acyl donor is far away from the carbonyl group, its effect on the enzymatic reaction is marginal (entry 5), as indicated by the similar catalytic performances, including the activity and regioselectivity, of PSL-C in the oleoylation and in the stearoylation. However, the reaction rate decreased substantially when the double bond conjugated with the carbonyl group, due to the resonance effect (entries 6–8). Surprisingly, although vinyl crotonate **3e** might be less hindered than vinyl methacrylate **3d** due to the presence of α-methyl group in the latter, the reaction rate with the latter as the acyl donor was higher than that with the former (entries 6 and 7). Up to date, the reason still remains unknown. Watanabe et al. obtained similar results in PSL-C-mediated synthesis of 6-esters of D-glucose: a yield of 39% was afforded with vinyl methacrylate as the acyl donor, a lower yield (11%) with vinyl acrylate, and no reaction with vinyl crotonate.[20] PSL-C-catalyzed benzoylation of FUdR was more difficult to perform (entries 9 and 10), owing to the steric and resonance effects of phenyl group. Prolonging arm length between phenyl and carbonyl would reduce the steric strain of the rigid phenyl group. As a result, vinyl cinnamate **3g** is easier to fit into the funnel-like active site of PSL-C as compared to vinyl benzoate **3f**, resulting in a higher reaction rate (entries 11 and 12).

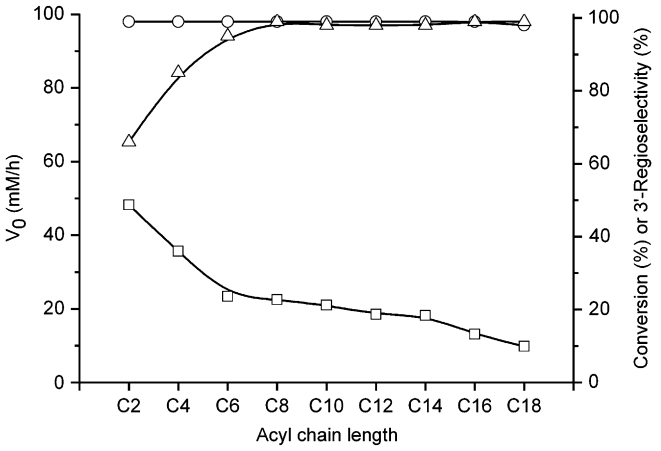


Figure 1. Effect of acyl chain length on PSL-C-catalyzed acylation of FUdR. The reaction was carried out at 40 °C, 250 rpm by adding 40 U PSL-C into anhydrous THF (2 mL) containing FUdR (0.04 mmol), vinyl esters (0.16 mmol) with $[C_4MMIm]PF_6$ (10 mg) as the additive. Symbols: V_0 (□); conversion (○); 3′-regioseletivity (△).

Table 2
Effect of the substituent or functional group present in the acyl donor on PSL-C-catalyzed acylation of FUdR[a]

Entry	Acyl donor	Temperature (°C)	Molar ratio[b]	Time (h)	Conversion[c] (%)	3′-Regioselectivity[d] (%)
1	**3a**	40	4	47	67	96
2		50	7	66	98	96
3	**3b**	40	4	47	10	83
4		50	7	90	41	82
5	**3c**	40	4	9	98	99
6	**3d**	40	4	23	99	77
7	**3e**	40	4	33	78	86
8		50	7	43	98	83
9	**3f**	40	4	107	58	87
10		50	7	66	99	88
11	**3g**	40	4	107	69	95
12		50	7	66	99	91

[a] The reaction was carried out at 250 rpm by adding 40 U PSL-C into anhydrous THF (2 mL) containing FUdR (0.04 mmol), vinyl esters with $[C_4MMIm]PF_6$ (10 mg) as the additive.
[b] Molar ratio of vinyl ester to FUdR.
[c] Determined by HPLC analysis using SB-C18 column.
[d] Defined as the ratio of the concentration of the desired product to that of all the products, and determined by HPLC analysis using SB-C18 column.

3. Conclusions

In summary, we have developed an efficient enzymatic approach for 3′-acylation of nucleosides and their analogs. The results revealed that the lower regioselectivities in PSL-C-catalyzed acylation of ribonucleosides **1f–1j** could be attributed to the unfavorable hydrogen bond interaction between 2′-hydroxyl group and phenolic hydroxyl group of tyrosine residue present in the alternate hydrophobic pocket of PSL-C. New interesting information regarding substrate–enzyme interactions was obtained from the study of enzyme substrate recognition in the acylation of nucleosides. These findings would help us in controlling the regioselectivity of the synthetically useful enzyme via chemical modification and protein engineering.

4. Experimental

4.1. General

P. cepacia lipase immobilized on ceramic (PSL-C) was from Amano Enzyme Inc., Japan. The specific esterification activity of PSL-C (730 U/g) was assayed according to a previous method.[14b] Floxuridine was purchased from Shanghai Hanhong Co., Ltd., China. 2′-Deoxyuridine, idoxuridine, β-thymidine, 5-bromo-2′-deoxyuridine, uridine, 5-fluorouridine, 5-methyluridine, 2′-fluoro-2′-deoxyuridine, and 1-β-D-arabinofuranosyluracil were bought from Tuoxin Biotechnology & Science Co., Ltd., China. 5-Iodouridine, 5-bromouridine, vinyl decanoate, vinyl laurate, vinyl stearate, vinyl methacylate, vinyl crotonate, vinyl benzoate were obtained from Sigma–Aldrich Co., USA. Vinyl butyrate, vinyl caproate, vinyl octanoate, vinyl myristate, vinyl palmitate, vinyl pivalate, vinyl 2-ethylhexanoate, and vinyl cinnamate were from TCI, Japan. All the chemicals mentioned above are of high purity (>99%). 1-Butyl-2,3-dimethylimidazolium hexafluorophosphate ($[C_4MMIm]PF_6$) was purchased from Lanzhou Institute of Chemical Physics, China. Oleic acid (90%) was from Alfa Aesar, USA. All other chemicals are of the highest purity commercially available. High performance liquid chromatography (HPLC) analysis was carried out in an Agilent 1200 chromatograph with a UV detector using Zorbax SB-C18 column (4.6 mm×250 mm, 5-Micron) under varying conditions depending on the specific substrate. The absorption wavelength for the analysis is 260 nm (2′-fluoro-2′-deoxyuridine), 261 nm (2′-deoxyuridine and uridine), 263 nm (1-β-D-arabinofuranosyluracil), 267 nm (β-thymidine and 5-methyluridine), 269 nm (FUdR and 5-fluorouridine), 279 nm (5-bromo-2′-deoxyuridine and 5-bromouridine), and 280 nm (idoxuridine and 5-iodouridine), respectively. The regioselectivity was defined as the ratio of the concentration of the desired product to that of all the products. The initial reaction rate (V_0) and the substrate conversion were calculated from the HPLC data.

4.2. General procedure for enzymatic acylation

In a typical experiment, a reaction mixture of FUdR (**1b**) (10 mg, 0.04 mmol), 4 equiv of vinyl ester and 10 mg $[C_4MMIm]PF_6$ in anhydrous THF (2 mL) was added to a sealed-cap vial (15 mL) containing PSL-C (40 U). The reaction was carried out at 40 °C, 250 rpm. Aliquots were withdrawn from the reaction mixture at specified time intervals, and then diluted by 25-fold with corresponding mobile phase prior to HPLC analysis.

4.3. Purification and structure determination of the desired ester derivatives

In a typical experiment, THF (8 mL) containing FUdR or its analogs (100 mg), 7 equiv of vinyl ester and PSL-C (300 mg) was incubated in a 50 mL Erlenmeyer shaking flask capped with a septum at 250 rpm and 50 °C. Upon completion of the reaction, the enzyme was filtered off, and the filtrate was concentrated in vacuo. The residue was separated and purified through column chromatography using petroleum ether (PE)/ethyl acetate (EA) or dichloromethane (DCM)/ethyl acetate (EA) as the eluent. The structures of ester derivatives of FUdR and its analogs were determined by ^{13}C NMR and ^{1}H NMR (Bruker DRX 400 MHz NMR spectrometer, Germany) at 100.5 and 400 MHz, respectively. All the ester derivatives of nucleosides are white solid.

4.3.1. 3′-O-Lauroyl-2′-deoxyuridine (**2a**)

R_f: 0.21 (PE/EA=4:7). ^1H NMR (DMSO-d_6) δ: 0.85 (t, J=8.0 Hz, 3H, $H_{12''}$), 1.24 (br s, 16H, $H_{4''}+H_{5''}+H_{6''}+H_{7''}+H_{8''}+H_{9''}+H_{10''}+H_{11''}$), 1.53 (t, J=8.0 Hz, 2H, $H_{3''}$), 2.24–2.27 (m, 2H, $H_{2'}$), 2.30–2.36 (m, 2H, $H_{2''}$), 3.61 (br s, 2H, $H_{5'}$), 3.98 (br s, 1H, $H_{4'}$), 5.19–5.23 (m, 2H, $H_{3'}$+OH), 5.68 (d, J=8.0 Hz, 1H, H_6), 6.16 (t, J=8.0 Hz, 1H, $H_{1'}$), 7.88 (d, J=8.0 Hz, 1H, H_5), 11.35 (s, 1H, H_3). ^{13}C NMR (DMSO-d_6) δ: 13.96 ($C_{12''}$), 22.11 ($C_{11''}$), 24.33 ($C_{3''}$), 28.39–29.36 ($C_{4''}+C_{5''}+C_{6''}+C_{7''}+C_{8''}+C_{9''}$), 31.30 ($C_{10''}$), 33.43 ($C_{2''}$), 36.85 ($C_{2'}$), 61.32 ($C_{5'}$), 74.67 ($C_{3'}$), 84.04 ($C_{4'}$), 84.84 ($C_{1'}$), 102.14 ($C_5$), 140.23 ($C_6$), 150.44 ($C_2$), 163.04 ($C_4$), 172.57 ($C_{1''}$).

4.3.2. 3′-O-Lauroyl-FUdR (**2b**)

R_f: 0.24 (PE/EA=3:2). ^1H NMR (DMSO-d_6) δ: 0.84 (br s, 3H, $H_{12''}$), 1.24 (br s, 16H, $H_{4''}+H_{5''}+H_{6''}+H_{7''}+H_{8''}+H_{9''}+H_{10''}+H_{11''}$), 1.53 (br s, 2H, $H_{3''}$), 2.27–2.36 (m, 4H, $H_{2''}+H_{2'}$), 3.64 (br s, 2H, $H_{5'}$), 4.00 (br s, 1H, $H_{4'}$), 5.22 (br s, 1H, $H_{3'}$), 6.15 (t, J=6.8 Hz, 1H, $H_{1'}$), 8.21 (d, J=7.2 Hz, 1H, H_6). ^{13}C NMR (DMSO-d_6) δ: 14.17 ($C_{12''}$), 22.32 ($C_{11''}$), 24.53 ($C_{3''}$), 28.57–29.18 ($C_{4''}+C_{5''}+C_{6''}+C_{7''}+C_{8''}+C_{9''}$), 31.51 ($C_{10''}$), 33.65 ($C_{2''}$), 37.11 ($C_{2'}$), 63.45 ($C_{5'}$), 74.84 ($C_{3'}$), 84.67 ($C_{4'}$), 85.19 ($C_{1'}$), 124.56, 124.90 ($C_6$), 139.16, 141.45 ($C_5$), 149.19 ($C_2$), 157.06, 157.33 ($C_4$), 172.94 ($C_{1''}$).

4.3.3. 3′-O-Lauroyl-β-thymidine (**2c**)

R_f: 0.21 (PE/EA=1:1). ^1H NMR ($CDCl_3$) δ: 0.88 (t, J=7.2 Hz, 3H, $H_{12''}$), 1.26 (br s, 16H, $H_{4''}+H_{5''}+H_{6''}+H_{7''}+H_{8''}+H_{9''}+H_{10''}+H_{11''}$), 1.61–1.64 (m, 2H, $H_{3''}$), 1.90 (s, 3H, H_7), 2.33–2.45 (m, 4H, $H_{2'}+H_{2''}$), 3.27 (br s, 1H, OH), 3.91 (br s, 2H, $H_{5'}$), 4.07 (br s, 1H, $H_{4'}$), 5.37 (t, J=2.8 Hz, 1H, $H_{3'}$), 6.29 (t, J=7.6 Hz, 1H, $H_{1'}$), 7.60 (s, 1H, H_6), 9.79 (s, 1H, H_3). ^{13}C NMR ($CDCl_3$) δ: 12.66 (C_7), 14.24 ($C_{12''}$), 22.79 ($C_{11''}$), 24.91 ($C_{3''}$), 29.23–29.71 ($C_{4''}+C_{5''}+C_{6''}+C_{7''}+C_{8''}+C_{9''}$), 32.01 ($C_{10''}$), 34.31 ($C_{2''}$), 37.44 ($C_{2'}$), 62.57 ($C_{5'}$), 74.71 ($C_{3'}$), 85.42 ($C_{4'}$), 85.86 ($C_{1'}$), 111.43 ($C_5$), 136.61 ($C_6$), 150.85 ($C_2$), 164.39 ($C_4$), 173.82 ($C_{1''}$).

4.3.4. 3′-O-Lauroyl-5-bromo-2′-deoxyuridine (**2d**)

R_f: 0.29 (PE/EA=3:2). ^1H NMR ($CDCl_3$) δ: 0.88 (t, J=7.2 Hz, 3H, $H_{12''}$), 1.26 (br s, 16H, $H_{4''}+H_{5''}+H_{6''}+H_{7''}+H_{8''}+H_{9''}+H_{10''}+H_{11''}$), 1.59–1.65 (m, 2H, $H_{3''}$), 2.33–2.50 (m, 5H, $H_{2'}+H_{2''}$+OH), 3.94–4.01 (m, 2H, $H_{5'}$), 4.12 (d, J=2.0 Hz, 1H, $H_{4'}$), 5.36 (d, J=4.4 Hz, 1H, $H_{3'}$), 6.30 (dd, J_1=14.0 Hz, J_2=6.0 Hz, 1H, $H_{1'}$), 8.26 (s, 1H, H_6), 9.14 (s, 1H, H_3). ^{13}C NMR ($CDCl_3$) δ: 14.36 ($C_{12''}$), 22.92 ($C_{11''}$), 25.04 ($C_{3''}$), 29.34–29.83 ($C_{4''}+C_{5''}+C_{6''}+C_{7''}+C_{8''}+C_{9''}$), 32.14 ($C_{10''}$), 34.41 ($C_{2''}$), 38.32 ($C_{2'}$), 62.75 ($C_{5'}$), 74.76 ($C_{3'}$), 85.83 ($C_{4'}$), 86.36 ($C_{1'}$), 97.38 ($C_5$), 140.39 ($C_6$), 149.89 ($C_2$), 159.19 ($C_4$), 173.90 ($C_{1''}$).

4.3.5. 3′-O-Lauroyl-idoxuridine (**2e**)

R_f: 0.36 (PE/EA=3:2). ^1H NMR ($CDCl_3$) δ: 0.88 (t, J=8.0 Hz, 3H, $H_{12''}$), 1.26 (br s, 16H, $H_{4''}+H_{5''}+H_{6''}+H_{7''}+H_{8''}+H_{9''}+H_{10''}+H_{11''}$), 1.61–1.64 (m, 2H, $H_{3''}$), 2.33–2.49 (m, 4H, $H_{2'}+H_{2''}$), 2.73 (br s, 1H, OH), 3.93–3.97 (m, 2H, $H_{5'}$), 4.12–4.13 (m, 1H, $H_{4'}$), 5.36–5.38 (m, 1H, $H_{3'}$), 6.26–6.29 (m, 1H, $H_{1'}$), 8.34 (s, 1H, H_6), 9.39 (s, 1H, H_3). ^{13}C NMR ($CDCl_3$) δ: 14.32 ($C_{12''}$), 22.87 ($C_{11''}$), 24.99 ($C_{3''}$), 29.30–29.98 ($C_{4''}+C_{5''}+C_{6''}+C_{7''}+C_{8''}+C_{9''}$), 32.09 ($C_{10''}$), 34.37 ($C_{2''}$), 38.29 ($C_{2'}$), 62.67 ($C_{5'}$), 68.75 ($C_5$), 74.72 ($C_{3'}$), 85.81 ($C_{4'}$), 86.35 ($C_{1'}$), 145.61 ($C_6$), 150.25 ($C_2$), 160.26 ($C_4$), 173.86 ($C_{1''}$).

*4.3.6. 3′-O-Lauroyl-uridine (**2f**)*

R_f: 0.26 (PE/EA=1:3). ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J=8.0 Hz, 3H, $H_{12''}$), 1.26 (br s, 16H, $H_{4''}+H_{5''}+H_{6''}+H_{7''}+H_{8''}+H_{9''}+H_{10''}+H_{11''}$), 1.60–1.65 (m, 2H, $H_{3''}$), 2.38–2.48 (m, 2H, $H_{2''}$), 3.80–3.93 (m, 2H, $H_{5'}$), 4.19 (br s, 1H, $H_{4'}$), 4.60 (t, J=8.0 Hz, 1H, $H_{2'}$), 5.27 (br s, 1H, $H_{3'}$), 5.74 (d, J=8.0 Hz, 1H, H_6), 5.83 (d, J=4.0 Hz, 1H, $H_{1'}$), 7.80 (d, J=8.0 Hz, 1H, H_5), 10.20 (s, 1H, H_3). ^{13}C NMR ($CDCl_3$) δ: 14.32 ($C_{12''}$), 22.89 ($C_{11''}$), 25.01 ($C_{3''}$), 29.37–29.83 ($C_{4''}+C_{5''}+C_{6''}+C_{7''}+C_{8''}+C_{9''}$), 32.11 ($C_{10''}$), 34.24 ($C_{2''}$), 61.99 ($C_{5'}$), 72.67 ($C_{3'}$), 73.29 ($C_{2'}$), 83.68 ($C_{4'}$), 90.75 ($C_{1'}$), 102.85 ($C_5$), 141.72 ($C_6$), 151.30 ($C_2$), 164.26 ($C_4$), 174.09 ($C_{1''}$).

*4.3.7. 3′-O-Lauroyl-5-fluorouridine (**2g**)*

R_f: 0.24 (DCM/EA=3:2). ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J=8.0 Hz, 3H, $H_{12''}$), 1.26 (br s, 16H, $H_{4''}+H_{5''}+H_{6''}+H_{7''}+H_{8''}+H_{9''}+H_{10''}+H_{11''}$), 1.59–1.63 (m, 2H, $H_{3''}$), 2.38–2.48 (m, 2H, $H_{2''}$), 3.83–3.95 (m, 2H, $H_{5'}$), 4.19 (d, J=4.0 Hz, 1H, $H_{4'}$), 4.52 (t, J=4.0 Hz, 1H, $H_{2'}$), 5.26 (br s, 1H, $H_{3'}$), 5.90 (d, J=4.0 Hz, 1H, $H_{1'}$), 8.08 (d, J=8.0 Hz, 1H, H_6), 10.38 (d, J=4.0 Hz, 1H, H_3). ^{13}C NMR ($CDCl_3$) δ: 14.32 ($C_{12''}$), 22.89 ($C_{11''}$), 24.98 ($C_{3''}$), 29.37–29.85 ($C_{4''}+C_{5''}+C_{6''}+C_{7''}+C_{8''}+C_{9''}$), 32.12 ($C_{10''}$), 34.19 ($C_{2''}$), 61.85 ($C_{5'}$), 72.64 ($C_{3'}$), 73.79 ($C_{2'}$), 83.76 ($C_{4'}$), 89.81 ($C_{1'}$), 125.34, 125.69 ($C_6$), 139.59, 141.94 ($C_5$), 149.97 ($C_2$), 157.74, 158.00 ($C_4$), 174.25 ($C_{1''}$).

*4.3.8. 3′-O-Lauroyl-5-methyluridine (**2h**)*

R_f: 0.27 (PE/EA=2:5). ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J=8.0 Hz, 3H, $H_{12''}$), 1.26 (br s, 16H, $H_{4''}+H_{5''}+H_{6''}+H_{7''}+H_{8''}+H_{9''}+H_{10''}+H_{11''}$), 1.59–1.66 (m, 2H, $H_{3''}$), 1.85 (s, 3H, H_7), 2.38–2.46 (t, 2H, $H_{2''}$), 3.81–3.94 (m, 2H, $H_{5'}$), 4.19 (d, J=4.0 Hz, 1H, $H_{4'}$), 4.63 (t, J=4.0 Hz, 1H, $H_{2'}$), 5.27 (t, J=4.0 Hz 1H, $H_{3'}$), 5.79 (d, J=4.0 Hz, 1H, $H_{1'}$), 7.57 (s, 1H, H_6), 10.11 (s, 1H, H_3). ^{13}C NMR ($CDCl_3$) δ: 12.61 (C_7), 14.35 ($C_{12''}$), 22.92 ($C_{11''}$), 25.04 ($C_{3''}$), 29.39–29.86 ($C_{4''}+C_{5''}+C_{6''}+C_{7''}+C_{8''}+C_{9''}$), 32.14 ($C_{10''}$), 34.27 ($C_{2''}$), 62.13 ($C_{5'}$), 72.66 ($C_{3'}$), 72.99 ($C_{2'}$), 83.60 ($C_{4'}$), 91.05 ($C_{1'}$), 111.41 ($C_5$), 137.55 ($C_6$), 151.45 ($C_2$), 164.57 ($C_4$), 174.15 ($C_{1''}$).

*4.3.9. 3′-O-Lauroyl-5-bromouridine (**2i**)*

R_f: 0.22 (PE/EA=3:2). ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J=8.0 Hz, 3H, $H_{12''}$), 1.25 (br s, 16H, $H_{4''}+H_{5''}+H_{6''}+H_{7''}+H_{8''}+H_{9''}+H_{10''}+H_{11''}$), 1.60–1.65 (m, 2H, $H_{3''}$), 2.40–2.48 (m, 2H, $H_{2''}$), 3.83–3.99 (m, 2H, $H_{5'}$), 4.22 (br s, 1H, $H_{4'}$), 4.64 (t, J=4.0 Hz, 1H, $H_{2'}$), 5.30 (t, J=4.0 Hz, 1H, $H_{3'}$), 5.91 (d, J=8.0 Hz, 1H, $H_{1'}$), 8.30 (s, 1H, H_6), 10.38 (s, 1H, H_3). ^{13}C NMR ($CDCl_3$) δ: 14.32 ($C_{12''}$), 22.88 ($C_{11''}$), 25.01 ($C_{3''}$), 29.36–29.84 ($C_{4''}+C_{5''}+C_{6''}+C_{7''}+C_{8''}+C_{9''}$), 32.11 ($C_{10''}$), 34.24 ($C_{2''}$), 61.72 ($C_{5'}$), 72.26 ($C_{3'}$), 73.94 ($C_{2'}$), 83.65 ($C_{4'}$), 90.43 ($C_{1'}$), 97.35 ($C_5$), 141.04 ($C_6$), 150.69 ($C_2$), 160.02 ($C_4$), 174.25 ($C_{1''}$).

*4.3.10. 3′-O-Lauroyl-5-iodouridine (**2j**)*

R_f: 0.30 (PE/EA=5:4). ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J=8.0 Hz, 3H, $H_{12''}$), 1.25 (br s, 16H, $H_{4''}+H_{5''}+H_{6''}+H_{7''}+H_{8''}+H_{9''}+H_{10''}+H_{11''}$), 1.60–1.66 (m, 2H, $H_{3''}$), 2.40–2.48 (m, 2H, $H_{2''}$), 3.83–3.4.01 (m, 2H, $H_{5'}$), 4.23 (br s, 1H, $H_{4'}$), 4.69 (t, J=4.0 Hz, 1H, $H_{2'}$), 5.34 (t, J=8.0 Hz, 1H, $H_{3'}$), 5.91 (d, J=4.0 Hz, 1H, $H_{1'}$), 8.39 (s, 1H, H_6), 10.41 (s, 1H, H_3). ^{13}C NMR ($CDCl_3$) δ: 14.33 ($C_{12''}$), 22.89 ($C_{11''}$), 25.03 ($C_{3''}$), 29.36–29.83 ($C_{4''}+C_{5''}+C_{6''}+C_{7''}+C_{8''}+C_{9''}$), 32.11 ($C_{10''}$), 34.27 ($C_{2''}$), 61.61 ($C_{5'}$), 69.28 ($C_5$), 72.07 ($C_{3'}$), 73.93 ($C_{2'}$), 83.49 ($C_{4'}$), 90.49 ($C_{1'}$), 146.19 ($C_4$), 151.04 ($C_2$), 161.08 ($C_6$), 173.85 ($C_{1''}$).

*4.3.11. 3′-O-Lauroyl-2′-fluoro-2′-deoxyuridine (**2k**)*

R_f: 0.32 (PE/EA=1:2). ^{1}H NMR (DMSO-d_6) δ: 0.85 (t, J=7.2 Hz, 3H, $H_{12''}$), 1.24 (br s, 16H, $H_{4''}+H_{5''}+H_{6''}+H_{7''}+H_{8''}+H_{9''}+H_{10''}+H_{11''}$), 1.51–1.56 (m, 2H, $H_{3''}$), 2.39 (t, J=7.2 Hz, 2H, $H_{2''}$), 3.56–3.61 (m, 2H, $H_{5'}$), 4.14 (t, J=3.2 Hz, 1H, $H_{4'}$), 5.18–5.25 (m, 1H, $H_{3'}$), 5.33–5.48 (m, 1H, $H_{2'}$), 5.70 (d, J=8.0 Hz, 1H, H_6), 5.95 (dd, J_1=21.6 Hz, J_2=3.2 Hz, 1H, $H_{1'}$), 7.86 (d, J=8.0 Hz, 1H, H_5). ^{13}C NMR (DMSO-d_6) δ: 14.34 ($C_{12''}$), 22.68 ($C_{11''}$), 24.91 ($C_{3''}$), 28.89–29.58 ($C_{4''}+C_{5''}+C_{6''}+C_{7''}+C_{8''}+C_{9''}$), 31.90 ($C_{10''}$), 33.68 ($C_{2''}$), 60.47 ($C_{5'}$), 70.09, 70.23 ($C_{3'}$), 82.18 ($C_{4'}$), 88.44, 88.78 ($C_{1'}$), 90.35, 92.24 ($C_{2'}$), 102.69 ($C_5$), 141.82 ($C_6$), 150.88 ($C_2$), 164.18 ($C_4$), 172.76 ($C_{1''}$).

*4.3.12. 3′-O-Lauroyl-1-β-D-arabinofuranosyluracil (**2m**)*

R_f: 0.23 (DCM/EA=1:1). ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J=7.2 Hz, 3H, $H_{12''}$), 1.26 (br s, 16H, $H_{4''}+H_{5''}+H_{6''}+H_{7''}+H_{8''}+H_{9''}+H_{10''}+H_{11''}$), 1.60–1.65 (m, 2H, $H_{3''}$), 2.38 (t, J=8.0 Hz, 2H, $H_{2''}$), 3.92–3.40 (m, 2H, $H_{5'}$), 4.10 (m, 1H, $H_{4'}$), 4.44 (br s, 1H, $H_{2'}$), 5.14 (br s, 1H, $H_{3'}$), 5.65 (d, J=8.0 Hz, 1H, H_6), 6.07 (d, J=3.6 Hz, 1H, $H_{1'}$), 7.79 (d, J=8.0 Hz, 1H, H_5), 9.82 (s, 1H, H_3). ^{13}C NMR ($CDCl_3$) δ: 14.32 ($C_{12''}$), 22.89 ($C_{11''}$), 24.96 ($C_{3''}$), 29.32–29.82 ($C_{4''}+C_{5''}+C_{6''}+C_{7''}+C_{8''}+C_{9''}$), 32.11 ($C_{10''}$), 34.25 ($C_{2''}$), 62.18 ($C_{5'}$), 73.41 ($C_{2'}$), 79.12 ($C_{3'}$), 83.79 ($C_{4'}$), 87.15 ($C_{1'}$), 100.93 ($C_5$), 142.54 ($C_6$), 150.56 ($C_2$), 164.78 ($C_4$), 173.64 ($C_{1''}$).

For the characterization data of other compounds, see Supplementary data.

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Supplementary data

HPLC analysis conditions, retention time, and characterization data are available. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.11.045.

References and notes

1. (a) Griffin, B. E.; Jarman, M.; Reese, C. B. *Tetrahedron* **1968**, *24*, 639–662; (b) Nishino, S.; Yamamoto, H.; Nagato, Y.; Ishido, Y. *Nucleosides Nucleotides Nucleic Acids* **1986**, *5*, 159–168.
2. (a) Kawaguchi, T.; Fukushima, S.; Hayashi, Y.; Nakano, M. *Pharm. Res.* **1988**, *5*, 741–744; (b) Nishizawa, Y.; Casida, J. E. *Biochem. Pharmacol.* **1965**, *14*, 1605–1619.
3. (a) Ferrero, M.; Gotor, V. *Chem. Rev.* **2000**, *100*, 4319–4347; (b) Park, H. G.; Do, J. H.; Chang, H. N. *Biotechnol. Bioprocess Eng.* **2003**, *8*, 1–8.
4. (a) Garcia, J.; Fernandez, S.; Ferrero, M.; Sanghvi, Y. S.; Gotor, V. *Tetrahedron: Asymmetry* **2003**, *14*, 3533–3540; (b) Diaz-Rodriguez, A.; Fernandez, S.; Lavandera, I.; Ferrero, M.; Gotor, V. *Tetrahedron Lett.* **2005**, *46*, 5835–5838; (c) Garcia, J.; Fernandez, S.; Ferrero, M.; Sanghvi, Y. S.; Gotor, V. *Nucleosides Nucleotides Nucleic Acids* **2003**, *22*, 1455–1457.
5. Lavandera, I.; Fernandez, S.; Magdalena, J.; Ferrero, M.; Grewal, H.; Savile, C. K.; Kazlauskas, R. J.; Gotor, V. *ChemBioChem* **2006**, *7*, 693–698.
6. (a) Sanghvi, Y. S.; Cook, P. D. In *Nucleosides and Nucleotides as Antitumor and Antiviral Agents*; Chu, C. K., Baker, D. C., Eds.; Plenum: New York, NY, 1993; pp 311–324; (b) De Clercq, E. *J. Clin. Virol.* **2004**, *30*, 115–133.
7. Chabner, B. A.; Ryan, D. P.; Paz-Area, L.; Garcia-Carbonero, R.; Calabresi, P. In *Goodman & Gilman's The Pharmacological Basis of Therapeutics*; Hardman, G., Limbird, L. E., Eds.; Mc-Graw Hill: New York, NY, 2001; pp 1389–1459.
8. Birnie, G. D.; Kroeger, H.; Heidelberger, C. *Biochemistry* **1963**, *2*, 566–572.
9. (a) De Clercq, E.; Field, H. J. *Br. J. Pharmacol.* **2006**, *147*, 1–11; (b) Halmos, T.; Moroni, P.; Antonakis, K.; Uriel, J. *Biochem. Pharmacol.* **1992**, *44*, 149–155; (c) Wang, J. X.; Sun, X.; Zhang, Z. R. *Eur. J. Pharm. Biopharm.* **2002**, *54*, 285–290.
10. Xia, Z. P.; Wiebe, L. I.; Miller, G. G.; Knaus, E. E. *Arch. Pharm.* **1999**, *332*, 286–294.
11. (a) Landowski, C. P.; Song, X. Q.; Lorenzi, P. L.; Hilfinger, J. M.; Amidon, G. L. *Pharm. Res.* **2005**, *22*, 1510–1518; (b) Landowski, C. P.; Vig, B. S.; Song, X. Q.; Amidon, G. L. *Mol. Cancer Ther.* **2005**, *4*, 659–667; (c) Vig, B. S.; Lorenzi, P. J.; Mittal, S.; Landowski, C. P.; Shin, H. C.; Mosberg, H. I.; Hilfinger, J. M.; Amidon, G. L. *Pharm. Res.* **2003**, *20*, 1381–1388.
12. (a) Li, X. F.; Lou, W. Y.; Smith, T. J.; Zong, M. H.; Wu, H.; Wang, J. F. *Green Chem.* **2006**, *8*, 538–544; (b) Li, X. F.; Zong, M. H.; Wu, H.; Lou, W. Y. *J. Biotechnol.* **2006**, *124*, 552–560; (c) Li, X. F.; Zong, M. H.; Yang, R. D. *J. Mol. Catal. B: Enzym.* **2006**, *38*, 48–53.
13. Wang, H.; Zong, M. H.; Wu, H.; Lou, W. Y. *J. Biotechnol.* **2007**, *129*, 689–695.

14. (a) Li, N.; Ma, D.; Zong, M. H. *J. Biotechnol.* **2008**, *133*, 103–109; (b) Li, N.; Zong, M. H.; Liu, X. M.; Ma, D. *J. Mol. Catal. B: Enzym.* **2007**, *47*, 6–12; (c) Li, N.; Zong, M. H.; Ma, D. *Eur. J. Org. Chem.* **2008**, *2008*, 5375–5378.
15. Four types of ionic liquid were examined as the additives in the enzymatic acylation, and the concentration of the additive was optimized. The information is available in Supplementary data.
16. Tuomi, W. V.; Kazlauskas, R. J. *J. Org. Chem.* **1999**, *64*, 2638–2647.
17. Pleiss, J.; Fischer, M.; Schmid, R. D. *Chem. Phys. Lipids* **1998**, *93*, 67–80.
18. Ozaki, S.; Yamashita, K.; Konishi, T.; Maekawa, T.; Eshima, M.; Uemura, A.; Ling, L. *Nucleosides Nucleotides* **1995**, *14*, 401–404.
19. Garcia, J.; Fernandez, S.; Ferrero, M.; Sanghvi, Y. S.; Gotor, V. *Tetrahedron Lett.* **2004**, *45*, 1709–1712.
20. Watanabe, T.; Matsue, R.; Honda, Y.; Kuwahara, M. *Carbohydr. Res.* **1995**, *275*, 215–220.