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Facile synthesis of novel mutual derivatives of nucleosides and pyrimidines by regioselectively chemo-enzymatic protocol

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Abstract—We established a facile regioselectively chemo-enzymatic synthesis procedure for the preparation of mutual derivatives of nucleosides and pyrimidines by sequential Markovnikov addition and acylation. Firstly, pyrimidine derivatives containing vinyl ester group were synthesized from pyrimidines and divinyl esters through Markovnikov addition catalyzed by K_2CO_3 in DMSO at 80 °C, and the yields were ranged from 50% to 87%. Then regioselective acylation of ribavirin and cytarabine with pyrimidine vinyl ester was catalyzed by CAL-B (immobilized lipase from *Candida antarctica*) in anhydrous acetone. Reaction conditions of enzymatic acylation including enzyme resource and solvents were optimized. A series of mutual derivatives of nucleosides and pyrimidines were synthesized successfully and characterized with NMR, IR, and HRMS. This chemo-enzymatic protocol involving sequential Markovnikov addition and acylation provided a novel way of synthesizing complicated functional compounds regioselectively which was hard to be achieved either by chemical or by enzymatic methods.

1. Introduction

Nucleosides are the most frequently used effective class of antiviral agents, with over twenty drugs currently approved for the treatment of viral diseases and a number of candidates in the clinical trials.[1,2] Consequently, the intense search for new nucleoside derivatives attracted extensive attention. For example, Mackman synthesized a novel nucleoside phosphonate which had anti-HIV activity.[3] Being bioactive molecules, pyrimidines are important components of the biological macromolecules, such as DNA and RNA. So introducing the pyrimidines into nucleoside derivatives may result in the discovery of a number of novel derivatives with potential antitumor and antiviral activities. The explosion of new approaches for their synthesis and most importantly, their selective synthesis is an interesting subject of organic and bioorganic chemistry.

Enzyme-catalyzed reactions are widely recognized as superior to conventional chemical methods in selective modification of polyfunctional substrates owing to mild reaction conditions, high catalytic efficiency, inherent selectivity, and simple downstream processing. Therefore, many research groups have paid much effort in the area of enzymatic synthesis of nucleoside derivatives.[4–9] Transesterification is the most common method to synthesize nucleoside derivatives. For instance, Hanson regioselectively synthesized the prodrug of Lobucavir by screening the solvents and enzymes.[10]

Many of the researches in this field, however, are focused on acylation reaction type which hardly provides more procedures for preparing complicated functional compounds. Therefore, the combination of the enzymatic reaction and diversified chemical reaction will be a smart choice. Gotor chemo-enzymaticly synthesized 3′- and 5′-carbazyl nucleoside derivatives.[11] Tanaka also synthesized antiviral carbocyclic nucleosides using rhizopus delemar lipase.[12] Addition reaction is one of the most basic types of reactions in organic synthesis. Exhilaratingly, we have found that some enzymes are able to catalyze general addition reactions such as Michael addition and Markovnikov addition.[13] It provided an easy way to introduce various functional groups into *N*-heterocycles including pyrimidine or purine. Our group has demonstrated a facile method to synthesis complex compounds containing the sugar moiety and heterocycles using the combination of enzyme-catalyzed addition and acylation reactions.[14]

Keywords: Chemo-enzymatic; Regioselectivity; Pyrimidine; Ribavirin; Cytarabine; Transesterification.
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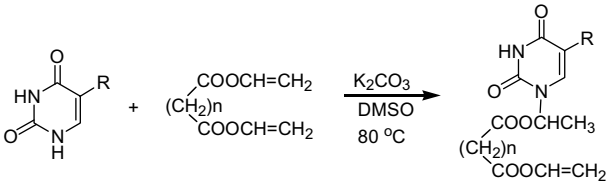
Here, we expected to regioselectively synthesize novel and complicated nucleoside derivatives containing bioactive pyrimidines. Two nucleosides including ribavirin and cytarabine, and three pyrimidines are selected as the starting substrates for the mutual derivatives of nucleoside and pyrimidines. We found the step of Markovnikov addition would carry out very well while using chemical catalyst such as K_2CO_3. Furthermore, reaction conditions for the base-catalyzed addition and enzymatic selective acylation were optimized, respectively. As a result, twelve mutual derivatives of nucleoside (ribavirin or cytarabine) and pyrimidines were regioselectively prepared in good or moderate yields using the chemo-enzymatic strategy combining Markovnikov addition and acylation.

2. Results and discussion

2.1. Influence of catalysts on Markovnikov addition

Markovnikov addition is one of the most basic types of reactions in organic synthesis which can facilely introduce various functional groups into *N*-heterocycles. Divinyl dicarboxylates are selected for the preparation of pyrimidines derivatives which will be subjected to the second-step transformations (Scheme 1). Markovnikov addition between thymidine and divinyl adipate was chosen as model reaction. Three kinds of bases were screened in order to find the most efficient one for the preparation of pyrimidine vinyl esters. And DMSO was selected as the reaction media because of that pyrimidine dissolve in it very well. The results are shown in Figure 1. The reaction between thymidine and vinyl ester was very sluggish without any catalyst, and only 3.5% yield was obtained after 2.5 h. Moderate yield ranged from 42% to 68% was observed when NaOH or [bmim]OH was used. K_2CO_3 showed the best catalytic activity and the yield reached to 87%. The optimization of the amount of K_2CO_3 showed that 0.1 equiv K_2CO_3 was enough for the efficient catalysis of Markovnikov addition (Fig. 2). There was no obvious difference as the dosage of K_2CO_3 increasing.

With optimal condition in hand, six pyrimidine vinyl esters were synthesized (Scheme 1). All the reaction underwent through Markovnikov addition smoothly with a high yield catalyzed by 10 mol % K_2CO_3 in DMSO. All the structure of the products was confirmed by IR, ^{1}H NMR, ^{13}C NMR, and HRMS. The results in



Scheme 1. Synthesis of pyrimidine vinyl esters (**1a**–**1f**).

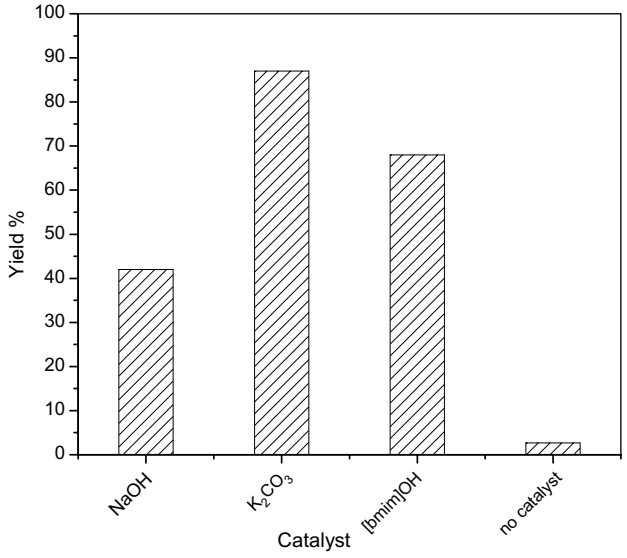


Figure 1. The influence of catalyst on the reaction. Conditions: thymidine (0.5 mmol), divinyl adipate (1 mmol); 0.05 mmol catalyst, or with no catalyst, DMSO 2 ml, 80 °C, 2.5 h. Determined by HPLC.

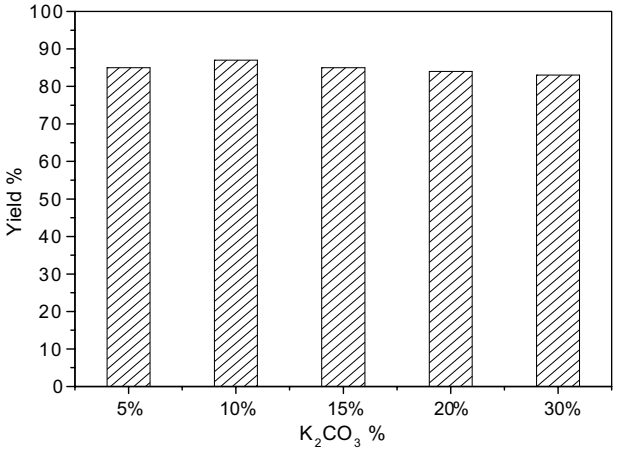


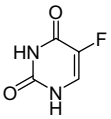
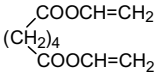
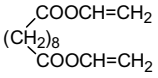
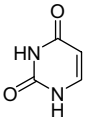
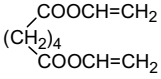
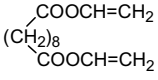
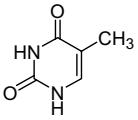
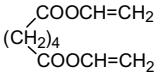
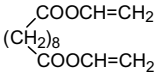
Figure 2. The influence of the amount of K_2CO_3 on the reaction. Conditions: thymidine (0.5 mmol), divinyl adipate (1 mmol); DMSO 2 ml, 80 °C, 2.5 h. Determined by HPLC.

Table 1 show that the structure of pyrimidines and the chain-length of the divinyl ester played a somewhat role in governing the reactivity of the Markovnikov addition. Generally, the electron withdrawing effect of substitution group was one of the main factors affecting the Markovnikov addition. The reactivity order decreased by the following order: thymidine, uracil, and fluorouracil. As the chain-length increased, the reaction yield increased slightly. The best result was obtained from the reaction between thymidine and divinyl sebacate (**1f**) and the isolated yield was 83%.

2.2. Influence of enzyme on the transesterification

Enzymes derived from various sources such as bacteria, yeast, and molds show different properties, including stability in organic solvent, activity, and specificity. In order to identify suitable enzymes in the transesterifica-

Table 1. The influence of substrate structure and the chain-length of divinyl esters on the reaction

Entry	Pyrimidine	Divinyl esters	Yield[a] (%)
1			**1a** 52
2			**1b** 64
3			**1c** 73
4			**1d** 75
5			**1e** 80
6			**1f** 83

Conditions: pyrimidine (8 mmol), divinyl ester (12 mmol); DMSO 10 ml, 80 °C, 2.5 h.
[a] Isolated yield.

tion of ribavirin with pyrimidine vinyl esters, eleven commercially available enzymes were employed in acetone at 50 °C. The screening results are presented and compared in Figure 3. PS-C had the lowest activity for this transesterification, and only provided product **2e** in 4.1% yield. Most enzymes, such as Acylase Amano, Alkaline protease from *Bacillus subtilisin*, and Lipozyme®, could not promote the reaction efficiently and the yields were ranged from 12% to 27%. However, CAL-B showed its unique activity with high yield up to 71.5%, and CCL (Lipase from *Candida cylindracea*) gave an acceptable yield of 53%. Thus, CAL-B was chosen as a catalyst in the further investigation.

2.3. Influence of organic solvents on the transesterification

Reaction media also play a crucial role in maintaining enzyme catalytic activity and stability. To optimize the reaction conditions for enzymatic transesterification of ribavirin, six polar solvents were screened considering the solubility of substrates (Fig. 4). The best results were obtained in anhydrous acetone and THF with yields of 71.5% and 57.3%, respectively. However, other four widely used solvents, including 1,4-dioxane, DMF, ethyl acetate, and pyridine were not suitable for the reaction. The yields were lower than 15%. Consequently, anhydrous acetone was employed as the reaction medium in the following synthesis.

2.4. Synthesis and characterization of ribavirin derivatives

After choosing appropriate enzymes and organic solvents, six ribavirin derivatives containing pyrimidine were synthesized under the optimal conditions as shown in Scheme 2. The products were purified by flash chromatography and analyzed by ^{13}C NMR and ^{1}H NMR spectra, and the acylation position of ribavirin derivatives was determined by ^{13}C NMR. According to the general strategy described by Yoshimoto et al. [15] acylation of a hydroxyl group of substrate results in a downfield shift of the peak corresponding to the *O*-acylated carbon and an upfield shift of the peak corresponding to the neighboring carbon. The ^{13}C NMR (DMSO-d_6) data of products **2a**–**2f** are shown in Table 2. For example, the chemical shift for C-5′ of product **2a** was downfield from 61.8 to 64.2 ppm, and that for C-4′ of product **2a** was upfield from 85.0 to 82.4 ppm. Therefore, based on the above analysis, we come to the conclusion that **2a** is the 5′-OH acylated product. The acylated position of other products **2b**–**2f** can be confirmed in a similar way.

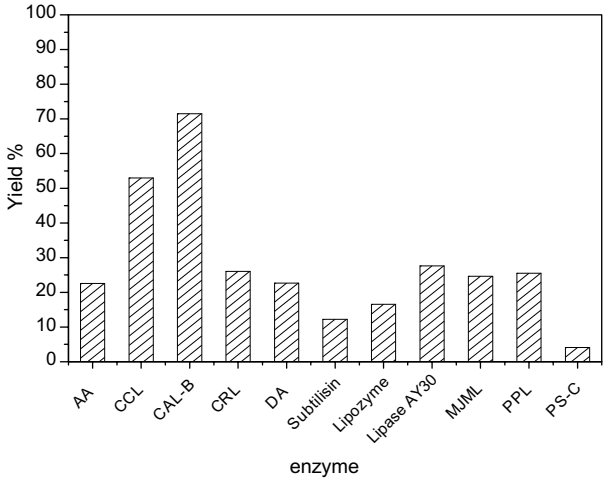


Figure 3. Influence of enzyme on transesterification. Conditions: ribavirin (0.5 mmol), thymidine vinyl adipate (1 mmol); 15 mg CAL-B, acetone 2 ml, 50 °C, 36 h. Determined by HPLC.

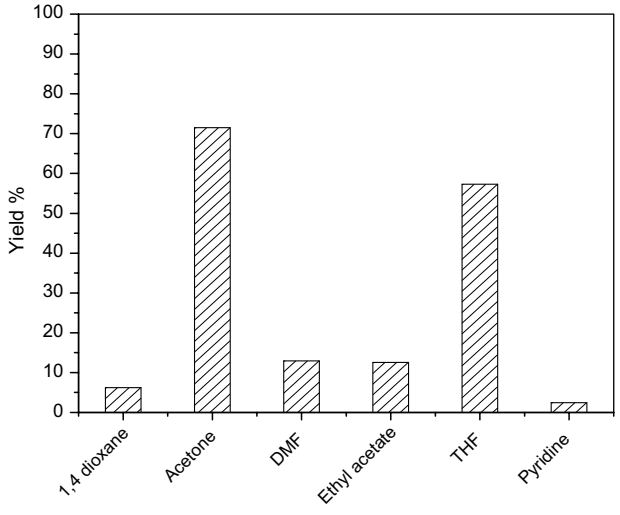
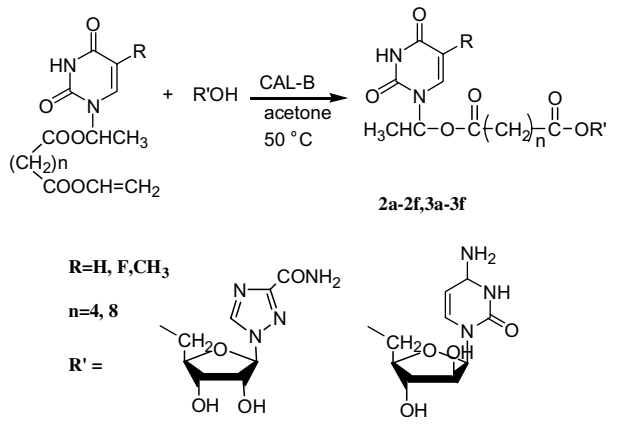
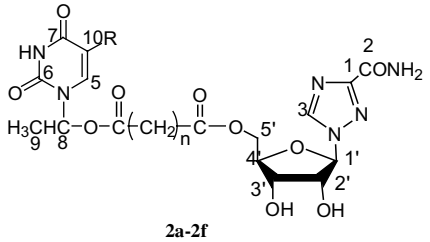


Figure 4. Influence of organic solvent on transesterification. Conditions: ribavirin (0.5 mmol), thymidine vinyl adipate (1 mmol); 15 mg CAL-B, organic solvent 2 ml, 50 °C, 36 h. Determined by HPLC.



Scheme 2. Synthesis of mutual derivatives of nucleosides and pyrimidines (**2a**–**2f**, **3a**–**3f**).

Table 2. Chemical shifts of ^{13}C NMR (DMSO-d_6) of ribavirin derivatives containing pyrimidine



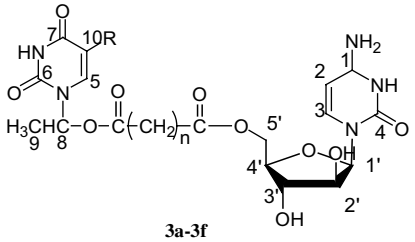
2a-2f

Carbon	Ribarvirin	2a	2b	2c	2d	2e	2f
1	157.8	160.8	161.1	160.9	160.9	161.0	161.0
2	145.4	146.0	146.1	146.1	146.1	146.1	146.1
3	160.9	158.1	158.1	158.1	158.1	158.1	158.1
1′	92.2	91.9	92.1	91.9	92.0	92.0	92.0
2′	75.0	74.6	74.8	74.7	74.8	74.7	74.8
3′	70.5	70.9	71.0	71.0	71.0	71.0	71.0
4′	85.0	82.2	82.4	82.3	82.3	82.3	82.3
5′	61.8	64.2	64.3	64.2	64.2	64.3	64.2
–CH_2		24.0	24.8	24.0	24.7	24.1	24.8
		24.1	24.9	24.1	24.9	24.2	24.9
		33.3	28.9	33.3	28.9	33.4	28.9
		33.4	28.9	33.4	28.9	33.5	28.9
			29.1		29.1		29.1
			29.1		29.1		29.1
			33.8		33.7		33.8
			33.9		33.9		33.9
–C=O		171.6	172.0	171.7	172.0	171.8	172.0
		173.1	173.4	173.1	173.4	173.2	173.4
10		141.7	141.8	102.9	102.9	110.8	110.7
5		125.2	125.3	141.0	141.0	136.3	136.3
6		149.0	149.1	150.3	150.4	150.4	150.4
7		157.4	157.5	163.6	163.6	164.3	164.3
8		75.6	75.7	75.5	75.4	75.3	75.2
9		18.9	19.0	19.2	19.2	19.3	19.2

2.5. Synthesis and characterization of cytarabine derivatives

Six cytarabine derivatives containing pyrimidine were also synthesized using the identical condition. The products were purified by flash chromatography and analyzed by ^{13}C NMR and ^{1}H NMR spectra. The chemical shift for C-5′ of product **3a** was downfield from 61.3 to 64.4 ppm, and that for C-4′ of product **3a** was upfield from 85.3 to 82.3 ppm. There were similar chemical shifts in the ^{13}C NMR of **3b**-**3f**. Characterization of products **3a**–**3f** by ^{13}C NMR spectra revealed that all the acylation was occurred at 5′-OH (Table 3). It demonstrated that the pyrimidine group had no influence on the regioselectivity of enzymatic acylation of ribavirin or cytarabine. The yields of mutual derivatives of nucleosides and pyrimidines are shown in Table 4.

Table 3. Chemical shifts of ^{13}C NMR (DMSO-d_6) of cytarabine derivatives containing pyrimidine



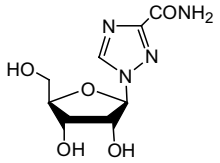
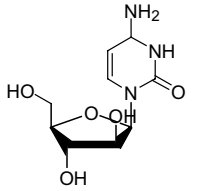
3a-3f

Carbon	Cytarabine	3a	3b	3c	3d	3e	3f
1	166.0	166.1	166.1	166.1	166.1	166.1	166.1
2	92.8	93.1	93.1	93.1	93.1	93.1	93.1
3	143.4	143.4	143.4	143.2	143.4	143.4	143.4
4	155.2	155.6	155.7	155.7	155.6	156.6	156.7
1′	86.3	86.7	86.7	86.7	86.7	86.7	86.7
2′	76.9	77.3	77.2	77.3	77.3	77.4	77.3
3′	75.3	74.8	74.8	74.9	74.8	74.8	74.8
4′	85.3	82.3	82.3	82.3	82.3	82.3	82.3
5′	61.3	64.4	64.2	64.3	64.2	64.2	64.2
–CH_2		24.0	24.7	24.4	24.7	24.2	24.7
		24.2	24.9	24.5	24.9	24.5	24.9
		33.3	28.9	33.4	28.9	33.8	28.8
		33.5	28.9	33.5	28.9	33.9	28.8
			29.0		29.0		29.0
			29.0		29.0		29.0
			33.7		33.7		33.8
			33.9		33.9		33.9
–C=O			171.8		171.9		171.9
			173.4		173.4		173.4
10		141.7	141.8	102.8	102.8	110.7	110.6
5		125.2	125.3	141.1	141.0	136.3	136.2
6		149.0	149.4	150.5	150.3	150.5	150.3
7		155.6	155.7	163.6	163.5	164.3	164.2
8		75.6	75.6	75.4	75.3	75.3	75.1
9		18.9	19.0	19.3	19.1	19.3	19.1

3. Conclusion

By optimizing the conditions of sequential two-step reactions, a new synthetic strategy of mutual derivatives bearing nucleosides and pyrimidine bioactive moieties via chemical Markovnikov addition and enzymatic acylation was established. Our results clearly demonstrated that the reaction between pyrimidine and vinyl ester could be efficiently promoted by 10 mol % K_2CO_3 in DMSO at 80 °C, and the further transesterification between ribavirin or cytarabine and fatty acid pyrimidine vinyl esters was carried out in acetone at 50 °C under CAL-B catalyst. A series of ribavirin and cytarabine derivatives were obtained with high regioselectivity. All the products were acylated at 5′-OH. The metho-

Table 4. Synthesis of mutual derivatives of nucleosides and pyrimidines

Entry	Pyrimidine vinyl esters	Nucleoside drug	Yield[a] (%)
1	**1a**		**2a** 41
2	**1b**		**2b** 22
3	**1c**		**2c** 26
4	**1d**		**2d** 30
5	**1e**		**2e** 45
6	**1f**		**2f** 41
7	**1a**		**3a** 22
8	**1b**		**3b** 28
9	**1c**		**3c** 21
10	**1d**		**3d** 31
11	**1e**		**3e** 33
12	**1f**		**3f** 37

Conditions: nucleoside drug (0.5 mmol), pyrimidine vinyl ester (1 mmol); 15 mg CAL-B, acetone 2 ml, 50 °C, 36 h.
[a] Isolated yield.

dology presented here should be readily applicable to the synthesis of other related nucleoside prodrugs.

4. Experimental

4.1. Materials

Lipozyme® (E.C. 3.1.1.1, an immobilized preparation of lipase from *Mucor miehei*, 42 μ/g), lipase from porcine pancreas (PPL) (E.C. 3.1.1.3, Type II, powder, 30–90 μ/mg), lipase from *C. cylindracea* (CCL) (E.C. 3.1.1.3, powder, 2.8 μ/mg) was purchased from Fluka. *C. antarctica* lipase acrylic resin (CAL-B) (E.C. 3.1.1.3, an immobilized preparation of lipase from *C. antarctica* on macroporous acrylic resin, 10,000 μ/g,) and lipase Type VII from *Candida rugosa* (CRL) (E.C. 3.1.1.3, powder, 706 μ/mg) were purchased from Sigma. Amano Lipase M, from *Mucor javanicus* (MJML) (E.C. 3.1.1.3, powder, 10 μ/mg), Lipase AY30 (E.C. 3.1.1.3, powder) was purchased from Acros. Alkaline protease from *B. subtilisin* (E.C. 3.4.21.14, a crude preparation of the alkaline serine protease, 100 μ/mg) was purchased from Wuxi Enzyme Co. Ltd (Wuxi, P.R. China). Acylase Amano (AA), D-aminoacylase Amano (DA), and lipase PS-C were purchased from Amano. Enzyme Inc. All the enzymes were used directly in commercially preparations without further purification. All the solvents were analytical grade and were dried by storing over activated 3 Å molecular sieves before use. All the other reagents were used as received.

4.2. Analytical methods

The process of reactions was monitored by TLC with Petroleum ether/EtOAc (1:2, v/v) for the pyrimidine vinyl eaters (**1a**–**1f**) and EtOAc/methanol/water (17:3:1, by vol) for the nucleosides derivatives (**2a**–**2f**, **3a**–**3f**). The ^{1}H NMR and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively, with TMS as internal standard using a Bruker AMX-500 MHz spectrometer. Chemical shifts were expressed in ppm and coupling constants (J) in Hz. IR spectra were measured with a Nicolet Nexus FTIR 670 spectrophotometer. Analytical HPLC was performed using an Agilent 1100 series with a reversed-phase Shim-Pack VP-ODS column (150 × 4.6 mm) and a UV detector (270 nm). Methanol/water (80:20, v/v) was used as a mobile phase, and the flow rate was 1 ml min^{-1}. HRMS were obtained on a Bruker 7-tesla FT-ICR MS equipped with an electrospray source (Billelica, MA, USA).

4.3. General procedure for the synthesis of pyrimidine vinyl esters

The reaction was initiated by adding K_2CO_3 (0.8 mmol) to 10 ml DMSO containing 8 mmol pyramidine and 12 mmol divinyl dicarboxylates, respectively. The suspension was kept at 80 °C and churned up for 2.5 h. The reaction was monitored by TLC (petroleum ether/ethyl acetate 1:2, v/v). The products were isolated by silica gel column chromatography with an eluent consisting of petroleum ether/ethyl acetate (1:2, v/v). Pyrimidine vinyl esters were characterized with IR, ^{1}H NMR, ^{13}C NMR, and HRMS or MS.

4.3.1. 1-(1-(5-Fluorouracil))-ethyl vinyl adipate (1a). The isolated yield of **1a** was 52%: white solid, mp 122–123 °C ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm): 1.53 (m, 7H), 2.35 (q, 2H, J = 6.41 Hz), 2.43 (t, 2H, J = 6.13 Hz), 4.64 (q, 1H, J = 1.5 Hz), 4.88 (q, 1H, J = 1.92 Hz), 6.76 (t, 1H, J = 6.25 Hz), 7.20 (q, 1H, J = 6.33 Hz), 8.19 (d, 1H, J = 6.90 Hz), 11.91 (m, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz, δ, ppm): 19.0, 23.9, 24.2, 33.2, 33.5, 75.6, 98.6, 125.4, 139.9, 141.7, 149.0, 157.5, 170.8, 171.6; IR (KBr, cm^{-1}): 2947, 1743, 1705, 1646, 1630, 1067, 1137, 1275; HRMS (EI) calcd for [M−C_2H_3O] = 285.0890, found: 285.0887.

4.3.2. 1-(1-(5-Fluorouracil))-ethyl vinyl sebacate (1b). The isolated yield of **1b** was 64%: white solid, mp 97–98 °C; ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm): 1.23 (m, 8H), 1.51 (m, 7H), 2.32 (q, 2H, J = 7.49 Hz), 2.45 (m, 2H), 4.64 (q, 1H, J = 1.48 Hz), 4.88 (q, 1H, J = 1.45 Hz), 8.19 (q, 1H, J = 1.18 Hz), 7.21 (q, 1H, J = 6.29 Hz), 6.77 (d, 1H, J = 6.93 Hz), 11.91 (m, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz, δ, ppm): 18.9, 24.6, 24.7, 28.9, 28.9, 29.1, 29.1, 33.5, 33.7, 75.6, 98.4, 125.4, 139.9; IR (KBr, cm^{-1}): 2918, 1743, 1708, 1648, 1628, 1068, 1155, 1298; HRMS (EI) calcd for [M−C_2H_3O] = 341.1518, found: 341.1513.

4.3.3. 1-(1-Uracil)-ethyl vinyl adipate (1c). The isolated yield of **1c** was 73%: white solid, mp 83–84 °C; ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm): 1.53 (m, 7H), 2.35 (d, 2H, J = 2.3 Hz), 2.43 (t, 2H, J = 6.25 Hz), 4.64 (q, 1H, J = 1.45 Hz), 4.88 (q, 1H, J = 1.45 Hz), 5.64 (d, 1H, J = 8.05 Hz), 6.77 (d, 1H, J = 6.25 Hz), 7.20 (q, 1H, J = 6.30 Hz), 7.76 (d, 1H, J = 8.05 Hz), 11.37 (s, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz, δ, ppm): 19.1, 23.8, 23.9, 33.1, 33.3, 75.4, 98.5, 102.8, 140.9, 141.7, 150.3, 163.5, 170.7, 171.6; IR (KBr, cm^{-1}):

2947, 1743, 1692, 1646, 1067, 1136, 1275; HRMS (EI) calcd for [M−C_2H_3O] = 267.0983, found: 267.0981.

4.3.4. 1-(1-Uracil)-ethyl vinyl sebacate (1d). The isolated yield of **1d** was 75%: white solid, mp 77–78 °C; ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm): 1.22 (m, 8H), 1.52 (m, 7H), 2.31 (d, 2H, J = 3.15 Hz), 2.40 (q, 2H, J = 7.40 Hz), 4.64 (q, 1H, J = 1.40), 4.88 (q, 1H, J = 1.45 Hz), 5.64 (q, 1H, J = 1.75 Hz), 6.77 (d, 1H, J = 6.25 Hz), 7.21 (q, 1H, J = 6.35 Hz), 7.77 (d, 1H, J = 8.05 Hz), 11.37 (s, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz, δ, ppm): 19.1, 24.5, 24.7, 28.9, 28.9, 29.1, 29.1, 33.5, 33.7, 75.4, 98.5, 102.8, 141.0, 141.8, 150.3, 163.5, 171.0, 171.9; IR (KBr, cm^{-1}): 2927, 1739, 1693, 1646, 1076, 1154, 1273; HRMS (EI) calcd for [M−C_2H_3O] = 323.1600, found: 323.1607.

4.3.5. 1-(1-Thymidine)-ethyl vinyl adipate (1e). The isolated yield of **1e** was 80%: white solid, mp 121–122 °C; ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm): 1.53 (m, 7H), 1.78 (d, 3H, J = 0.95 Hz), 2.35 (d, 2H, J = 5.35 Hz), 2.43 (q, 2H, J = 3.55 Hz), 4.64 (q, 1H, J = 1.50 Hz), 4.88 (q, 1H, J = 1.55 Hz), 6.79 (d, 1H, J = 6.25 Hz), 7.20 (q, 1H, J = 6.35 Hz), 7.64 (d, 1H, J = 1.1 Hz), 11.35 (s, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz, δ, ppm): 15.5, 19.1, 23.8, 24.0, 33.1, 33.4, 75.2, 98.5, 110.7, 136.2, 141.7, 150.3, 164.2, 170.7, 171.6; IR (KBr, cm^{-1}): 2950, 1749, 1691, 1648, 1142, 1279; HRMS (EI) calcd for [M−C_2H_3O] = 281.1138, found: 281.1137.

4.3.6. 1-(1-Thymidine)-ethyl vinyl sebacate (1f). The isolated yield of **1f** was 83%: white solid, mp 105–106 °C; ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm): 1.23 (m, 8H), 1.52 (m, 7H), 1.79 (s, 3H), 2.31 (q, 2H, J = 7.15 Hz), 2.40 (t, 2H, J = 7.15 Hz), 4.63 (q, 1H, J = 1.15 Hz), 4.88 (q, 1H, J = 1.20 Hz), 6.79 (q, 1H, J = 6.10 Hz), 7.21 (q, 1H, J = 6.35 Hz), 7.64 (s, 1H), 11.35 (s, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz, δ, ppm): 12.5, 19.1, 24.5, 24.7, 28.8, 28.8, 28.9, 29.00, 33.5 33.8, 75.1, 98.4, 110.6, 136.2, 141.7, 150.3, 164.2, 170.9, 171.8; IR (KBr, cm^{-1}): 2930, 1745, 1700, 1647, 1156, 1274, HRMS (EI) calcd for [M−C_2H_3O] = 337.1757, found: 337.1753.

4.4. General procedure for the enzymatic acylation of ribavirin and cytarabine

The reaction was initiated by adding 15 mg CAL-B to 2 ml acetone containing 0.5 mmol ribavirin or cytarabine, 1 mmol pyrimidine vinyl esters (**1a**–**1f**), respectively. The suspension was kept at 50 °C and shaken at 200 rpm for 36 h. The reaction was monitored by TLC (ethyl acetate/methanol/water (17:3:1, by vol)). The reaction was terminated by filtering off the enzyme, and the filtrate was concentrated under reduced pressure. The products were isolated by silica gel column chromatography with an eluent consisting of ethyl acetate/methanol/water (17:3:1, by vol).

4.4.1. 5′-*O*-[1-(1-(5-Fluorouracil))-ethyl vinyl adipate]-ribavirin (2a). The isolated yield of **2a** was 41%: white gum; ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm): 11.38 (s, 1H), 8.81 (s, 1H), 8.17 (q, 1H), 7.84 (s, 1H), 7.63 (s, 1H), 6.76 (d, 1H, J = 6.26 Hz), 5.89 (d, 1 H, J = 2.74 Hz), 5.70 (s, 1H), 5.39 (s, 1H), 4.32 (m, 3H), 4.09 (m, 2H), 2.33 (m, 4H); 1.50 (m, 7H), ^{13}C NMR (DMSO-d_6, 125 MHz, δ, ppm): 18.9, 24.0, 24.1, 33.3, 33.4, 64.2, 70.9, 74.61, 75.6, 82.2, 91.9, 125.2, 141.7, 146.0, 149.0, 157.4, 158.1, 160.8, 171.6, 173.1; IR (KBr, cm^{-1}): 3430 (υ_{OH}), 1724, 1697 ($\upsilon_{C=O}$); HRMS (ESI) calcd for [M+Na]$^+$ = 551.1495, found: 551.1508.

4.4.2. 5′-*O*-[1-(1-(5-Fluorouracil))-ethyl vinyl sebacate]-ribavirin (2b). The isolated yield of **2b** was 22%: white gum; ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm): 8.81 (d, 1H, J = 5.68 Hz), 8.18 (d, 1H, J = 6.86 Hz), 7.83 (s, 1H), 7.63 (s, 1H), 6.76(q, 1H, J = 1.07 Hz), 5.89 (s, 1H), 5.73 (s, 1H), 5.34 (s, 1H), 4.34 (m, 3H), 4.09 (m, 2H), 2.30 (m, 4H); 1.50 (m, 7H), 1.19 (s, 8H); ^{13}C NMR (DMSO-d_6, 125 MHz, δ, ppm): 19.0, 24.8, 24.9, 28.9, 28.9, 29.1, 29.1, 33.8, 33.9, 64.3, 71.0, 74.8, 75.7, 82.4, 92.1, 125.3, 141.8, 146.1, 149.1, 157.5, 161.1, 172.0, 173.4; IR (KBr, cm^{-1}): 3421 (υ_{OH}), 1719, 1687 ($\upsilon_{C=O}$); HRMS (ESI) calcd for [M+Na]$^+$ = 607.2124, found: 607.2134.

4.4.3. 5′-*O*-[1-(1-Uracil)-ethyl vinyl adipate]-ribavirin (2c). The isolated yield of **2c** was 26%: white gum; ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm): 11.36 (s, 1H), 8.81 (s, 1H), 7.85 (s, 1H), 7.76 (q, 1H), 7.64 (s, 1H), 6.78 (q, 1H, J = 6.26 Hz), 5.89 (d, 1H, J = 2.74 Hz), 5.67 (m, 1H), 5.64 (q, 1H, J = 2.2), 5.39 (s, 1H), 4.32 (m, 3H), 4.09 (m, 2H), 2.33 (m, 4H), 1.50 (m, 7H); ^{13}C NMR (DMSO-d_6, 125 MHz, δ, ppm): 14.6, 19.2, 24.0, 24.1, 33.3, 33.4, 64.2, 71.0, 74.7, 75.5, 82.3, 92.0, 102.9, 141.0, 146.1, 150.3, 158.1, 160.9, 163.6, 171.7, 173.1; IR (KBr, cm^{-1}): 3418 (υ_{OH}), 1723, 1684 ($\upsilon_{C=O}$); HRMS (ESI) calcd for [M+Na]$^+$ = 533.1601, found: 533.1603.

4.4.4. 5′-*O*-[1-(1-Uracil)-ethyl vinyl sebacate]-ribavirin (2d). The isolated yield of **2d** was 30%: white gum; ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm): 11.37 (s, 1H), 8.81 (s, 1H), 7.84 (s, 1H), 7.77 (d, 1H, J = 8.05 Hz), 7.63 (s, 1H), 6.77 (d, 1H, J = 6.25 Hz), 5.89 (d, 1H, J = 2.8 Hz), 5.68 (d, 1H, J = 5.1 Hz), 5.38 (d, 1H, J = 5.9 Hz), 4.34 (m, 3H), 4.07 (m, 2H), 2.30 (m, 4H), 1.50 (m, 7H), 1.18 (m, 8H); ^{13}C NMR (DMSO-d_6, 125 MHz, δ, ppm): 19.2, 24.7, 24.9, 28.9, 28.9, 29.1, 29.1, 33.7, 33.8, 64.2, 71.0, 74.8, 75.4, 82.3, 92.0, 102.9, 141.0,146.1, 150.4, 158.1, 160.9, 163.6, 172.0, 173.4. IR (KBr, cm^{-1}): 3437 (υ_{OH}), 1740, 1684 ($\upsilon_{C=O}$); HRMS (ESI) calcd for [M+Na]$^+$ = 589.2212, found: 589.2229.

4.4.5. 5′-*O*-[1-(1-Thymidine)-ethyl vinyl adipate]-ribavirin (2e). The isolated yield of **2e** was 45%: white gum; ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm): 11.35 (s, 1H), 8.81 (s, 1H), 7.85 (s, 1H), 7.64 (s, 2H), 6.78 (d, 1H, J = 6.10 Hz), 5.88 (s, 1H), 5.68 (s, 1H, 2′-OH), 5.37 (s, 1H), 4.32 (t, 3H), 4.05 (m, 2H), 2.30 (m, 4H); 1.78 (s, 3H), 1.50 (m, 7H); ^{13}C NMR (DMSO-d_6, 125 MHz, δ, ppm): 12.6, 19.3, 24.1, 24.2, 33.4, 33.5, 64.3, 71.0, 74.7, 75.3, 82.3, 92.0, 110.8, 136.3, 146.1, 150.4, 158.1, 161.0, 164.3, 171.8, 173.2. IR (KBr, cm^{-1}): 3420 (υ_{OH}), 1739, 1696 ($\upsilon_{C=O}$); HRMS (ESI) calcd for [M+Na]$^+$ = 547.1747, found: 547.1759.

4.4.6. 5′-*O*-[1-(1-Thymidine)-ethyl vinyl sebacate]-ribavirin (2f). The isolated yield of **2f** was 41%: white gum; ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm): 11.34 (s, 1H), 8.81 (d, 1H, J = 5.56 Hz), 7.84 (s, 1H), 7.64 (d, 2H, J = 5.43 Hz), 6.79 (d, 1H, J = 6.23 Hz), 5.88 (d, 1H, J = 2.71 Hz), 5.69 (s, 1H), 5.38 (s, 1H), 4.32 (m, 3H), 4.07 (m, 2H), 2.29 (m, 4H), 1.79 (s, 3H), 1.48 (m, 7H), 1.17 (m, 8H); ^{13}C NMR (DMSO-d_6, 125 MHz, δ, ppm): 12.6, 19.2, 24.8, 24.9, 28.9, 28.9, 29.1, 29.1, 33.8, 33.9, 64.2, 71.0, 74.8, 75.2, 82.3, 92.0, 110.7, 136.3, 146.1, 150.4, 158.1, 161.0, 164.3, 172.0, 173.4; IR (KBr, cm^{-1}): 3408 (υ_{OH}), 1740, 1685($\upsilon_{C=O}$). HRMS (ESI) calcd for $[M+Na]^+$ = 603.2376, found: 603.2385.

4.4.7. 5′-*O*-[1-(1-(5-Fluorouracil))-ethyl vinyl adipate]-cytarabine (3a). The isolated yield of **3a** was 22%: white gum; ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm): 8.18 (d, 1H, J = 6.83 Hz), 7.04 (s, 1H), 7.13 (s, 1H), 7.46 (d, 1H, J = 7.24), 6.77 (d, 1H, J = 6.12 Hz), 6.08 (d, 1H, J = 3.69 Hz), 5.67 (d, 1H, J = 7.41 Hz), 5.56 (s, 2H), 4.26 (m, 1H), 4.19 (m, 1H), 3.90 (s, 1H), 3.89 (m, 1H), 3.87 (m, 1H), 2.33 (m, 4H); 1.51 (m, 7H); ^{13}C NMR (DMSO-d_6, 125 MHz, δ, ppm): 18.9, 24.0, 24.2, 33.3, 33.5, 64.4, 74.8, 75.6, 77.3, 82.3, 86.7, 93.1, 125.2, 125.5, 139.9, 141.7, 143.4, 149.0, 155.6, 166.1, 171.6, 173.1. IR (KBr, cm^{-1}): 3410 (υ_{OH}), 1745, 1686 ($\upsilon_{C=O}$). HRMS (ESI) calcd for $[M+Na]^+$ = 550.1550, found: 550.1556.

4.4.8. 5′-*O*-[1-(1-(5-Fluorouracil))-ethyl vinyl sebacate]-cytarabine (3b). The isolated yield of **3b** was 28%: white gum; ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm): 8.13 (d, 1H, J = 6.84 Hz), 7.02 (s, 1H), 7.17 (s, 1H), 7.46 (d, 1H, J = 7.41 Hz), 6.76 (d, 1H, J = 6.18 Hz), 6.08 (d, 1H, J = 3.79 Hz), 5.68 (d, 1H, J = 7.43 Hz), 5.59 (s, 2H), 4.29 (m, 1H), 4.16 (m, 1H), 3.90 (s, 1H), 3.88 (m, 1H), 3.87 (m, 1H), 2.29 (m, 4H); 1.51 (m, 7H), 1.16 (m, 8H); ^{13}C NMR (DMSO-d_6, 125 MHz, δ, ppm): 19.0, 24.7, 24.9, 28.9, 28.9, 29.0, 29.0, 33.7, 33.9, 64.2, 74.8, 75.6, 77.2, 82.3, 86.7, 93.1, 125.0, 125.3, 140.0, 141.8, 143.4, 149.4, 155.7, 166.1, 171.8, 173.4. IR (KBr, cm^{-1}): 3417 (υ_{-OH}), 1733 ($\upsilon_{-C=O}$), 1648 ($\upsilon_{-CH=CH2}$); HRMS (ESI) calcd for $[M+Na]^+$ = 606.2166, found: 606.2182.

4.4.9. 5′-*O*-[1-(1-Uracil)-ethyl vinyl adipate]-cytarabine (3c). The isolated yield of **3c** was 21%: white gum; ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm): 11.37(s, 1H), 7.76 (d, 1H, J = 7.34 Hz), 7.14 (s, 1H), 7.05 (s, 1H), 7.47 (d, 1H, J = 6.21 Hz), 6.75 (q, 1H, J = 5.32 Hz), 6.06 (d, 1H, J = 2.15 Hz), 5.63(m, 2H), 5.55 (s, 2H), 4.26 (m, 1H), 4.16 (m, 1H), 3.90 (s, 1H), 3.89 (m, 1H), 3.87 (m, 1H), 2.29 (m, 4H); 1.50 (m, 7H), ^{13}C NMR (DMSO-d_6, 125 MHz, δ, ppm): 19.3, 24.4, 24.5, 33.4, 33.5, 34.0, 51.7, 64.3, 75.4, 77.3, 82.3, 86.7, 93.1, 102.8, 129.0, 141.1, 143.2, 150.5, 155.7, 163.6, 166.1, 171.5, 173.8; IR (KBr, cm^{-1}): 3426 (υ_{-OH}), 3206 (υ_{-NH2}), 1734 ($\upsilon_{-C=O}$), 1651 ($\upsilon_{-CH=CH2}$). MS: $[M+H]^+$ = 508.

4.4.10. 5′-*O*-[1-(1-Uracil)-ethyl vinyl sebacate]-cytarabine (3d). The isolated yield of **3d** was 31%: white gum; ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm): 11.34 (s, 1H), 7.75 (d, 1H, J = 7.97 Hz), 7.12 (s, 1H), 7.02 (s, 1H), 7.46 (d, 1H, J = 7.41 Hz), 6.76 (q, 1H, J = 5.67 Hz), 6.08 (d, 1H, J = 3.23 Hz), 5.64 (m, 2H), 5.56 (s, 2H), 4.28 (m, 1H), 4.16 (m, 1H), 3.89 (s, 1H), 3.86 (m, 1H), 3.85 (m, 1H), 2.29 (m, 4H), 1.51 (m, 7H),1.19 (s, 8H); ^{13}C NMR (DMSO-d_6, 125 MHz, δ, ppm): 19.1, 24.7, 24.9, 28.9, 28.9, 29.0, 29.0, 33.7, 33.9, 64.2, 74.8, 75.3, 77.3, 82.3, 86.7, 93.1, 102.8, 141.0, 143.7, 150.3, 155.6, 163.5, 166.1, 171.9, 173.4; HRMS (ESI) calcd for $[M+Na]^+$ = 588.2275, found: 588.2276.

4.4.11. 5′-*O*-[1-(1-Thymidine)-ethyl vinyl adipate]-cytarabine (3e). The isolated yield of **3e** was 33%: white gum; ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm): 11.35 (s, 1H), 7.63 (d, 1H, J = 0.97 Hz), 7.11 (s, 1H), 7.02 (s, 1H), 7.46 (q, 1H, J = 1.16 Hz), 6.79 (q, 1H, J = 6.19 Hz), 6.08 (d, 1H, J = 3.68 Hz), 5.67 (d, 1H, J = 7.43 Hz), 5.57 (s, 2H), 4.28 (m, 1H), 4.19 (m, 1H), 3.95 (s, 1H), 3.91 (m, 1H),3.87 (m, 1H), 2.33 (m, 4H); 1.79 (s, 3H), 1.52 (m, 7H); ^{13}C NMR (DMSO-d_6, 125 MHz, δ, ppm): 12.9, 19.3, 24.2, 24.5, 33.8, 33.9, 64.2, 74.8, 75.3, 77.4, 82.3, 87.7, 93.1, 110.7, 136.3, 143.4, 150.5, 156.6, 164.3, 166.1, 172.3, 173.8; IR (KBr, cm^{-1}): 3421 (υ_{-OH}), 3213 (υ_{-NH2}), 1735, 1686 ($\upsilon_{-C=O}$), 1648 ($\upsilon_{-CH=CH2}$) MS: $[M+H]^+$ = 522.0.

4.4.12. 5′-*O*-[1-(1-Thymidine)-ethyl vinyl sebacate]-cytarabine (3f). The isolated yield of **3f** was 37%: white gum; ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm): 11.32 (s, 1H), 7.65 (s, 1H), 7.12 (s, 1H), 7.03 (s, 1H), 7.46 (d, 1H, J = 7.35), 6.79 (q, 1H, J = 6.20Hz), 6.08 (d, 1H, J = 3.36 Hz), 5.66 (d, 1H, J = 7.35 Hz), 5.56 (s, 2H), 4.29 (m, 1H), 4.17 (m, 1H), 3.90 (s, 1H), 3.87(m, 1H), 3.86 (m, 1H), 2.29 (m, 4H); 1.79 (s, 3H), 1.50 (m, 7H), 1.21 (s, 8H); ^{13}C NMR (DMSO-d_6, 125 MHz, δ, ppm): 12.5, 19.1, 24.7, 24.9, 28.8, 28.8, 29.0, 29.0, 33.8, 33.9, 64.2, 74.8, 75.1, 77.3, 82.3, 86.7, 93.1, 110.6, 136.2, 143.4, 150.3, 155.7, 164.2, 166.1, 171.9, 173.4; IR (KBr, cm^{-1}): 3421 (υ_{-OH}), 3213 (υ_{-NH2}), 1735, 1684 ($\upsilon_{-C=O}$), 1647 ($\upsilon_{-CH=CH2}$); HRMS (ESI) calcd for $[M+Na]^+$ = 602.2423, found: 602.2433.

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References and notes

1. Mansour, T. S.; Storer, R. *Curr. Pharm. Des.* **1997**, *3*, 227.
2. Bergmana, A. M.; Kuipera, C. M.; Voorn, D. A.; Comijna, E. M.; Myhrenb, F.; Sandvoldb, M. L.; Hendriksc, H. R.; Petersa, G. J. *Biochem. Pharmacol.* **2004**, 503.
3. Boojamra, C. G.; Mackman, R. L.; Markevitch, D. Y.; Prasad, V.; Ray, A. S.; Douglas, J.; Grant, D.; Kim, C. U.; Cihlar, T. *Bioorg. Med. Chem. Lett.* **2008**, *11*, 125. doi:10.1016/j.bmcl.2007.

4. Sugimoto, H.; Nakanishi, E.; Yamauchi, F.; Yasumura, T.; Inomata, K. *Polymer* **2005**, *46*, 10800.
5. Duncan, R. *Nat. Rev. Cancer* **2006**, *6*, 688.
6. Bridiau, N.; Taboubi, S.; Marzouki, N.; Legoy, M. D.; Maugard, T. *Biotechnol. Prog.* **2006**, *22*, 326.
7. Sun, X. F'.; Wang, N.; Wu, Q.; Lin, X. F. *Biotechnol. Lett.* **2004**, 1019.
8. Li, X.; Wu, Q.; Lv, D. S.; Lin, X. F. *Bioorg. Med. Chem.* **2006**, 3377.
9. Liu, B. K.; Wang, N.; Chen, Z. C.; Wu, Q.; Lin, X. F. *Bioorg. Med. Chem. Lett.* **2006**, 3769.
10. Hanson, R. L.; Shi, Z.; Brzozowski, D. B. *Bioorg. Med. Chem.* **2000**, *8*, 2681.
11. Lavandera, I.; Fernandez, S.; Magdalena, J.; Ferrero, M.; Kazlauskas, R. J.; Gotor, V. *Chembiochem* **2005**, *6*, 1381.
12. Julia, M.; Susana, F. *J. Org. Chem.* **1998**, *63*, 8873.
13. Wu, W. B.; Wang, N.; Xu, J. M.; Qi, W.; Lin, X. F. *Chem. Commun.* **2005**, 2348.
14. Xia, A. H.; Wu, Q.; Liu, B. K.; Lin, X. F. *Enzyme Microb. Technol.* **2007**. doi:10.1016/j.enzmictec.2007.12.001.
15. Yoshimoto, K.; Itatani, Y.; Tsuda, Y. *Chem. Pharm. Bull.* **1980**, *28*, 2065.