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# Controllable selective synthesis of a polymerizable prodrug of cytarabine by enzymatic and chemical methods

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**Abstract**—Selectivity of enzymatic and chemical methods for transesterifications of cytarabine with divinyl dicarboxylates was described. Catalysis by lipase acrylic resin from *Candida antarctica* (CAL-B) in acetone facilitated the single step synthesis of polymerizable 5′-*O*-acyl cytarabine derivatives in high yields, while the use of alkaline protease from *Bacillus subtilis* (subtilisin) in pyridine afforded the mixture products of 5′-*O*-acyl and 4-*N*-acyl cytarabine derivatives. Interestingly, polymerizable 4-*N*-acyl cytarabine vinyl derivatives can be selectively prepared by chemical transesterification in dioxane. The obtained series of cytarabine derivatives would be useful for a significant monomer for a polymeric anticancer drug.


Nucleosides are fundamental building blocks for biological systems, with a wide range of biological activity.[1] Especially, pyrimidine nucleosides analogs have shown particular antiviral and antitumor activities.[2,3] Therefore, extensive modifications have been made to the heterocyclic base or the sugar moiety to improve biological activity. In recent years, the search for new nucleoside derivatives using clean, simple, and efficient enzymatic methodology has been paid much attention by organic chemists.[4,5] Ferrero and Gotor[6] have reviewed the utility of biocatalysts for the modification of nucleosides, carbocyclic nucleosides, and C-nucleosides.

Cytarabine [1-β-D-arabino furanosylcytosine (Ara-C)] is an anticancer nucleoside analog widely used for the treatment of both acute and chronic myeloblastic leukemias.[7,8] Appropriately designed cytarabine prodrugs, such as acyloxyalkyl esters and phosphoramidates, are reported to increase nucleoside triphosphate levels in nucleoside kinase-deficient cell lines.[9,10] However, these prodrugs are usually 5′-hydroxyl derivatives and mostly obtained by conventional chemical methods using process of blocking/deblocking step for the 4-amino has higher activity than 5′-hydroxyl.[11] Moreover, few modified cytarabine prodrugs can be used for the preparation of macromolecular nucleoside anticancer drugs, which can effectively control the rate of the drug release and be administered at low doses, increasing the therapeutic benefit.[12]

In this letter, a facile enzymatic selective synthesis of vinyl cytarabine esters under mild conditions is described, and the chemo- and regioselectivities by chemical and enzymatic methods for cytarabine modification with divinyl dicarboxylates are reported. 4-*N*-acyl and 5′-*O*-acyl cytarabine derivatives have been prepared through two different methods, respectively.

The transesterification of cytarabine **1** with divinyl dicarboxylates (**2a**–**d**, $n$ = 2, 4, 7, and 8) in dioxane reflux was first carried out for 24 h, giving the product **3a**–**d** in 35%, 28%, 22%, and 33% yields, respectively. Then, four commercially available enzymes, alkaline protease from *Bacillus subtilis* (Subtilisin), lipase from porcine pancreas (PPL), lipase acrylic resin from *Candida antarctica* (CAL-B), and immobilized lipase from *Mucor miehei* (Lipozyme®), in a predominantly anhydrous medium were chosen in catalyzing the transesterification for comparison. The use of CAL-B or Lipozyme® in acetone facilitated the single step synthesis of 5′-*O*-acyl cytarabine vinyl esters. While catalyzed by PPL in acetone or THF, no product was detected. Interestingly, the transesterification catalyzed by subtilisin in pyridine gave the mixture of 5′-*O*-acyl cytarabine vinyl esters and 4-*N*-acyl cytarabine vinyl esters. The synthesis route is shown in Scheme 1.

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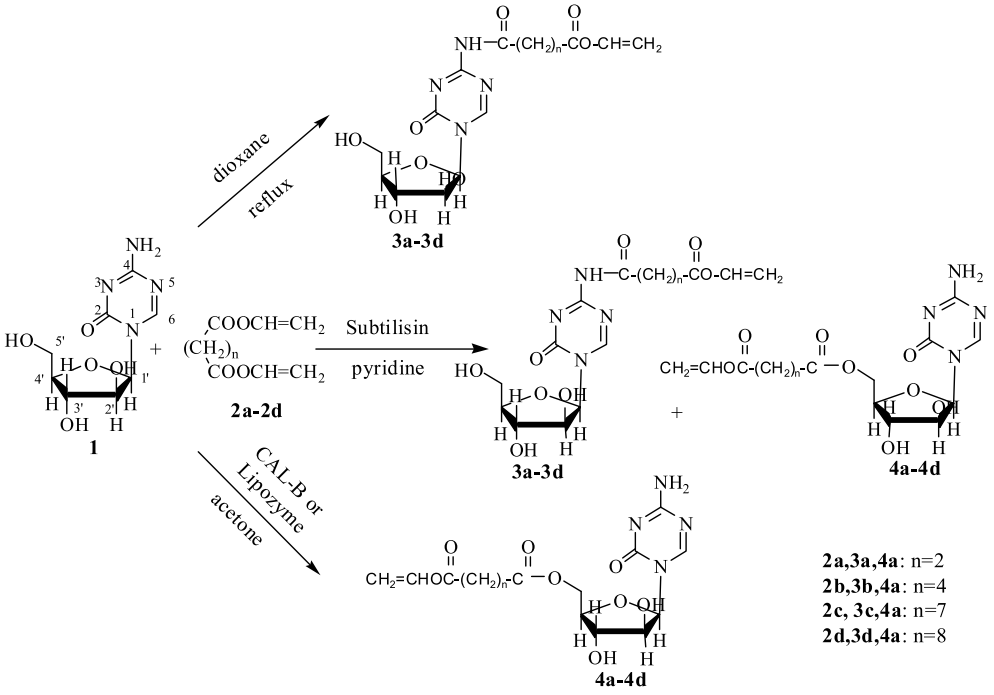


**Scheme 1.** Synthesis of 4-*N*-acyl and 5′-*O*-acyl cytarabine vinyl esters.

A typical enzymatic experimental procedure for the synthesis of **3d** and **4d** is illustrated in Scheme 1. The reaction was initiated by adding 10 mg/ml subtilisin to 25 ml anhydrous pyridine containing 0.5 g (2 mmol) cytarabine and 8 mmol divinylsebacate. The suspension was kept at 50 °C and shaken at 200 rpm. The reaction was terminated by filtering off the enzyme, and the filtrate was concentrated under reduced pressure. Formation of cytarabine ester was confirmed by TLC. The products were separated by silica gel chromatography with an eluent consisting ethyl acetate/methanol/water (20/1/0.8, v/v/v). The obtained cytarabine esters were characterized by $^{1}$H NMR, $^{13}$C NMR (Bruker DMX 500), IR (Nicolet Nexus FTIR670), and MS (Bruker esquire 3000 ESI-MS).[13] The yields of the cytarabine derivatives are summarized in Table 1.

To confirm whether pyridine has a catalytic effect in the reaction, the transesterification of cytarabine with divinyl dicarboxylates was carried out in pyridine in the absence of enzyme. No 4-*N*-vinylsebacate or 5′-*O*-vinylsebacate was detected by TLC and HPLC even after 3 days.[14] Several solvents were screened for the subtilisin-catalyzed synthesis of 4-*N*-vinylsebacate cytarabine (**3d**) and 5′-*O*-vinylsebacate cytarabine (**4d**). The transesterifications were carried out in pyridine, THF, DMF, and dioxane at 50 °C. On the basis of HPLC analysis results, subtilisin showed high activity in pyridine and very low activity in THF, DMF, and dioxane. The high activity of subtilisin in pyridine is also verified in our previous study and other groups' research.[15–18] Similar experiments were carried out for CAL-B in acetone, THF,

**Table 1.** Chemo- and regioselective enzymatic acylation of cytarabine

| Entry | Acyl agent | Enzyme | Solvent | *t* (h) | Yields of 5′-*O* (%) | Yields of 4-*N* (%) |
|---|---|---|---|---|---|---|
| 1[a] | **2a** | Subtilisin | Pyridine[c] | 12 | 58 | 14 |
| 2[b] | **2b** | Subtilisin | Pyridine | 72 | 46 | 12 |
| 3[b] | **2c** | Subtilisin | Pyridine | 96 | 55 | 22 |
| 4[b] | **2d** | Subtilisin | Pyridine | 120 | 54 | 23 |
| 5[b] | **2a** | CAL-B | Acetone | 24 | 52 | ND |
| 6[a] | **2b** | CAL-B | Acetone | 48 | 57 | ND |
| 7[b] | **2c** | CAL-B | Acetone | 96 | 68 | ND |
| 8[b] | **2d** | CAL-B | Acetone | 96 | 65 | ND |
| 9[a] | **2a** | Lipozyme® | Acetone | 24 | 12 | ND |
| 10[a] | **2b** | Lipozyme® | Acetone | 48 | 8 | ND |
| 11[a] | **2c** | Lipozyme® | Acetone | 72 | 15 | ND |
| 12[a] | **2d** | Lipozyme® | Acetone | 72 | 18 | ND |

ND, not detected.
[a] Percentage of compounds calculated by HPLC after workup.[14]
[b] Isolated yields by silica gel chromatography.
[c] Solvents dried over 4 Å molecular sieves for 24 h prior to use.

**Table 2.** Chemical shifts of $^{13}C$ NMR (DMSO-$d_6$) of cytarabine and cytarabine esters

| Carbon | 1 | 3a | 3b | 3c | 3d | 4a | 4b | 4c | 4d |
|---|---|---|---|---|---|---|---|---|---|
| 2 | 155.5 | 155.0 | 155.0 | 155.0 | 155.0 | 155.5 | 155.6 | 155.6 | 155.5 |
| 4 | 166.0 | 162.3 | 163.6 | 162.7 | 162.7 | 166.1 | 166.1 | 166.1 | 166.1 |
| 5 | 92.8 | 94.5 | 94.5 | 94.7 | 94.7 | 93.0 | 93.1 | 93.0 | 93.0 |
| 6 | 143.4 | 146.9 | 147.0 | 147.1 | 147.1 | 143.3 | 143.3 | 143.3 | 143.3 |
| 1′ | 86.3 | 87.0 | 87.2 | 87.4 | 87.4 | 86.7 | 86.7 | 86.6 | 86.6 |
| 2′ | 76.9 | 76.4 | 79.8 | 76.6 | 76.6 | 77.2 | 77.2 | 77.2 | 77.2 |
| 3′ | 75.3 | 75.2 | 75.3 | 75.1 | 75.1 | 74.8 | 74.8 | 74.8 | 74.8 |
| 4′ | 85.3 | 85.8 | 86.2 | 86.2 | 86.2 | 82.1 | 82.3 | 82.2 | 82.3 |
| 5′ | 61.3 | 61.2 | 61.3 | 61.5 | 61.4 | 64.7 | 64.3 | 64.2 | 64.2 |
| –$CH_2$ | | 31.2 | 36.3 | 36.8 | 36.8 | 28.8 | 33.5 | 33.8 | 33.9 |
| | | 29.8 | 33.1 | 33.5 | 33.5 | 28.7 | 33.1 | 33.4 | 33.5 |
| | | | 24.5 | 28.8 | 29.0 | | 24.2 | 28.7 | 28.9 |
| | | | 23.9 | 28.7 | 29.0 | | 23.9 | 28.6 | 28.8 |
| | | | | 28.6 | 28.9 | | | 28.4 | 28.8 |
| | | | | 24.9 | 28.8 | | | 24.8 | 28.7 |
| | | | | 24.5 | 24.9 | | | 24.4 | 24.9 |
| | | | | | 24.5 | | | | 24.5 |
| C=O | | 174.1 | 174.2 | 174.3 | 174.3 | 172.2 | 173.1 | 173.3 | 173.3 |
| | | 170.9 | 179.0 | 170.9 | 170.9 | 170.0 | 170.7 | 170.9 | 170.9 |
| –CH=$CH_2$ | | 141.6 | 141.7 | 141.7 | 141.7 | 141.6 | 141.7 | 141.7 | 141.7 |
| | | 98.4 | 98.5 | 98.5 | 98.5 | 98.7 | 98.6 | 98.5 | 98.5 |

isopropyl ether, and dioxane, and the best result was obtained in acetone.

The amino group in cytarabine has higher activity in chemical synthesis of polymerizable cytarabine monomers; as a result, the substitution happened at N-4 position and formed products **3a**–**d**. Compared with the chemical synthesis, the transesterification catalyzed by CAL-B or Lipozyme® showed high chemo- and regioselectivities among amino group and three hydroxyl groups, and the substitution happened at C-5′ position of cytarabine without any blocking/deblocking steps and formed products **4a**–**d**. To be mentioned is that CAL-B was used three times without a noticeable loss of activity. Interestingly, we also observed the mixture of acylation at amino group of N-4 position and at the primary hydroxyl group of C-5′ position when catalyzed by subtilisin in pyridine. However, the yields of products **4a**–**d** were higher than those of products **3a**–**d**, and this result indicated that the primary hydroxyl group of C-5′ position had higher activity than the amino group of N-4 position in the subtilisin-catalyzed transesterification.

The position of acylation was determined by $^1H$ NMR and $^{13}C$ NMR, according to the general strategy described by Yoshimoto et al.[19] 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. Characterization of the products **4a**–**d** by $^{13}C$ NMR, $^1H$ NMR, and IR revealed that vinyl cytarabine esters were substituted at C-5′ position of cytarabine, and the products **3a**–**d** were substituted at N-4 position. The $^{13}C$ NMR data of products are summarized in Table 2.

In summary, the chemo- and regioselectivities by chemical and enzymatic methods for cytarabine modification with divinyl dicarboxylates were described. 4-*N*-Acyl and 5′-*O*-acyl cytarabine derivatives can be prepared by chemical transesterification in dioxane reflux and lipase-catalyzed transesterification in acetone, respectively. However, the transesterification catalyzed by alkaline protease from *B. subtilis* in pyridine afforded the mixture products of 5′-*O*-acyl and 4-*N*-acyl cytarabine derivatives. The obtained vinyl cytarabine esters derivatives would be useful as a significant monomer for polymeric anticancer drug. The investigation of its chemical polymerization and further application as a polymeric drug or functional material are in progress.

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13. $^1$H NMR spectrum of **3d** (DMSO-$d_6$, $\delta$, ppm): 10.78 (s, 1H, –NH), 8.05 (d, 1H, $J$ = 7.35 Hz, 6-H) 7.22 (m, 2H, $=CH_2$, 5-H), 6.05 (d, 1H, $J$ = 3.65 Hz, 1′-H), 5.47 (br, 2H, 2′-OH, 3′-OH), 5.05 (t, 1H, $J$ = 5.1 Hz, 5′-OH), 4.88 (d, 1H, $J$ = 13.95 Hz, $CH_2=$), 4.64 (d, 1H, $J$ = 6.2 Hz, $CH_2=$), 4.06 (m, 1H, 2′-H), 3.92 (m, 1H, 4′-H), 3.82 (m, 1H, 3′-H), 3.61 (t, 2H, $J$ = 5.2 Hz, 5′-H), 2.40 (m, 4H, $–CH_2–$), 1.54 (m, 4H, $–CH_2–$), 1.26 (m, 8H, $–CH_2–$). IR spectrum of **3d** (KBr, cm$^{-1}$): 3333 (br, –OH, –NH), 1755 (–C=O), 1690 (–NHC=O), 1651 ($–CH=CH_2$). MS of **3d**: 476.0 ($M_1$+$Na^+$). $M_1$ corresponding exactly to 4-*N*-vinyl-sebacate-cytarabine' molecular weight. $^1$H NMR spectrum of **4d** (DMSO-$d_6$, $\delta$ , ppm): 7.46 (d, 1H, $J$ = 7.43 Hz, 6-H), 7.22 (dd, 1H, $J$ = 6.26 Hz, $J$ = 13.5 Hz, –CH=), 7.12 (br, 1H, $–NH_2$), 7.02 (br, 1H, $–NH_2$), 6.08 (d, 1H, $J$ = 3.72 Hz, 1′-H) 5.66 (d, 1H, $J$ = 7.42 Hz, 5-H), 5.55 (m, 2H, 2′-OH, 3′-OH), 4.89 (dd, 1H, $J$ = 13.95 Hz, $J$ = 1.32 Hz, $CH_2=$), 4.65 (d, 1H, $J$ = 6.28 Hz, $J$ = 1.3 Hz, $CH_2=$), 4.29 (m, 1H, 2′-H), 4.19 (m, 1H, 4′-H), 3.96 (m, 1H, 3′-H), 3.90 (m, 1H, 5′-H), 3.87 (m, 1H, 5′-H), 2.40 (m, 2H, $–CH_2–$), 2.32 (m, 2H, $–CH_2–$), 1.53 (m, 4H, $–CH_2–$), 1.25 (m, 8H, $–CH_2–$). IR spectrum of **4d** (KBr, cm$^{-1}$): 3342 (–OH), 3228 ($–NH_2$), 1758, 1773 (–C=O), 1644 ($–CH=CH_2$). MS of **4d**: 476.0 ($M_2$+$Na^+$). $M_2$ corresponding exactly to 5′-*O*-vinlysebacate-cytarabine' molecular weight.
14. HPLC analysis: Samples were dissolved in methanol and analyzed by HPLC using a Kromasil 100 Å C-18 column (5 μm, 4.6 × 200 mm) in an Agilent 1100 system, eluted with acetonitrile/water (40/60, by vol.) at 1 ml/min and UV detection was carried out at 280 nm.
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