Enzymatic Reactivity and Anti-tumor Activity of 1-(β-D-Arabinofuranosyl)-2-thiocytosine Derivatives

Takeo KAWAGUCHI,*,[a] Tomohisa ICHIKAWA,[a] Tetsuya HASEGAWA,[a] Mineo SANEYOSHI,[b] Toshiyuki WAKAYAMA,[c] Hisatoyo KATO,[c] Ayako YUKITA,[c] and Toshiyuki NAGATA[c]

Department of Pharmaceutical Sciences, Josai University,[a] 1–1 Keyakidai, Sakado, Saitama 320–0295, Japan, Department of Biological Sciences, Teikyo University of Science and Technology,[b] 2525 Yatsuzawa, Uenohara, Yamanashi 409–0133, Japan, and Tsukuba Research Laboratory, Toagosei Co., Ltd.,[c] 2 Ohkubo, Tsukuba, Ibaraki 300–2611, Japan.
Received July 19, 1999; accepted December 18, 1999

Sixteen derivatives of 1-(β-D-arabinofuranosyl)-2-thiocytosine (araSC), including five 5′-esters, three 3′-esters, five N^4-amides and three 5′-phosphodiesters, were synthesized and their reactivity to mouse tissue homogenates, including plasma, liver and intestine, and antitumor activity in mice bearing P388 cells were measured. The ester derivatives had a potent effect on the enzyme systems while the amide and phosphodiester derivatives were less active. The reactivity of ester derivatives was highly dependent on their chemical structure. The reactivity of amides and phosphodiester derivatives on mouse plasma and intestinal homogenate was also dependent on the chemical structure, although their action on intestinal enzymes was very similar. Two of eight ester derivatives showed considerable antitumor activity *in vivo*, although they also showed serious toxicity indicated by a weight loss in the mice. Four out of five amides and two out of three phosphodiesters showed antitumor activity, and two were highly effective (>200% in T/C, the ratio of the mean survival time of the treated group to that of the control group) with only a very slight weight loss.

Key words 1-(β-D-arabinofuranosyl)-2-thiocytosine; enzymatic regeneration; P388; tissue homogenate; prodrug

1-(β-D-Arabinofuranosyl)-2-thiocytosine (araSC) is a sulfur-substituted derivative of arabinosylcytosine (araC).[1)] Our previous studies demonstrated that it exhibited potent cytotoxicity and unique cytokinetics.[2)] The cytotoxic effect of araSC against several tumor cell lines *in vitro* is comparable or slightly less than that of araC, although the antitumor activity *in vivo* is dependent on many factors and can be modified by changes in retention, enzymatic and chemical stability, physicochemical properties, *etc*. To improve its activity as an antitumor agent, and obtain information to help in further possible modifications, sixteen derivatives of araSC were synthesized. Each carried a different chemical moiety with an ester, amide or phosphodiester linkage. These derivatives can behave as prodrugs, regenerating the parent compound, araSC, following enzymatic and/or chemical hydrolysis in the body. The effects of these compounds were examined in mice bearing P388 leukemia cells, and the relationship between the activity and enzymatic reactivity to tissue homogenates will be discussed.

Materials and Methods

Chemicals AraC was purchased from Yamasa Co. (Choshi, Japan). AraSC was prepared from uridine by the method reported by Ruyle and Shen.[1)] AraSC derivatives (Table 1) were synthesized from araSC hydrochloride, purified and identified by the following methods.

General Procedure for Obtaining 5′-*O*-Acyl Derivatives (1—5) AraSC (3.0 mmol) was dissolved in 10 ml *N*,*N*-dimethylacetamide, and the appropriate acyl chloride (3.3 mmol) was added dropwise. After stirring at room temperature for 2 d, water (0.5 ml) was added to the solution which was then concentrated under reduced pressure. The residue was partitioned between ethyl acetate and aq. $NaHCO_3$ and the organic layer was separated, washed twice with NaCl-saturated water, dried with Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to afford 5′-*O*-acyl araSC. This material was dissolved in methanol, and aq. HCl was added. Excess HCl and solvent were removed under reduced pressure. The residue was recrystallized from ethyl acetate–hexane.

1-(5′-*O*-Octanoyl-β-D-arabinofuranosyl)-2-thiocytosine (**1**): Yield: 77%, ^{1}H-NMR (DMSO-d_6+D_2O) δ: 0.85 (3H, t, *J*=6.8 Hz, CH_3), 1.25 (8H, br s, CH_2×4), 1.54 (2H, br t, $COCH_2C\underline{H}_2$), 2.33 (2H, t, *J*=7.8 Hz, $COCH_2$), 3.96 (1H, s, 3′-H), 4.08 (1H, m, 4′-H), 4.21 (1H, dd, *J*=4.9, 11.72 Hz, 5′-H), 4.25 (1H, d, 2′-H), 4.38 (1H, dd, *J*=4.9, 11.7 Hz, 5′-H), 6.47 (1H, d, *J*=7.8 Hz, 5-H), 6.73 (1H, d, *J*=3.4 Hz, 1′-H), 7.89 (1H, d, *J*=7.8 Hz, 6-H). FAB-MS *m/z*: 386 (55, MH^+), 259 (3, M^+−base), 128 (100, base+$2H^+$).

1-(5′-*O*-Lauroyl-β-D-arabinofuranosyl)-2-thiocytosine (**2**): Yield: 71%, mp 111—113 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.85 (3H, t, *J*=6.8 Hz, CH_3), 1.23 (16H, br s, CH_2×8), 1.53 (2H, m, $COCH_2C\underline{H}_2$), 2.33 (2H, t, *J*=7.3 Hz, $COCH_2$), 3.95 (1H, m, 3′-H), 4.07 (1H, ddd, *J*=2.0, 4.4, 7.8 Hz, 4′-H), 4.21 (1H, dd, *J*=4.4, 11.7Hz, 5′-H), 4.25 (1H, m, 2′-H), 4.37 (1H, dd, *J*=7.8, 11.7 Hz, 5′-H), 6.42 (1H, d, *J*=7.8 Hz, 5-H), 6.74 (1H, d, *J*=2.9 Hz, 1′-H), 7.88 (1H, d, *J*=7.8 Hz, 6-H). FAB-MS *m/z*: 442 (32, MH^+), 315 (2, M^+−base), 183 (3, $C_{11}H_{23}CO^+$), 128 (100, base+$2H^+$). *Anal.* Calcd for $C_{21}H_{35}N_3O_5SHCl$: C, 52.76; H,7.59; N,8.79. Found: C, 52.51; H, 7.49; N, 8.79.

1-(5′-*O*-Myristoyl-β-D-arabinofuranosyl)-2-thiocytosine (**3**): Yield: 95%, mp 124—125 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.86 (3H, t, *J*=6.8 Hz, CH_3), 1.23 (20H, br s, CH_2×10), 1.54 (2H, m, $COCH_2C\underline{H}_2$), 2.33 (2H, t, *J*=7.3 Hz, $COCH_2$), 3.95 (1H, m, 3′-H), 4.07 (1H, ddd, *J*=1.5, 4.4, 7.8 Hz, 4′-H), 4.21 (1H, dd, *J*=4.4, 11.7 Hz, 5′-H), 4.25 (1H, dd, *J*=1.5, 2.9 Hz, 2′-H), 4.37 (1H, dd, *J*=7.8, 11.7 Hz, 5′-H), 6.40 (1H, d, *J*=7.8 Hz, 5-H), 6.75 (1H, d, *J*=2.9 Hz, 1′-H), 7.87 (1H, d, *J*=7.8 Hz, 6-H). FAB-MS *m/z*: 470 (5, MH^+), 343 (1, M^+−base), 211 (5, $C_{13}H_{27}CO^+$), 128 (40, base+$2H^+$), 112 (100, cytosine). *Anal.* Calcd for $C_{23}H_{39}N_3O_5SHCl$: C, 54.58; H, 7.97; N, 8.30. Found: C, 54.22; H, 7.95; N, 8.25.

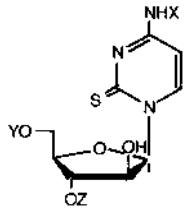
1-(5′-*O*-Palmitoyl-β-D-arabinofuranosyl)-2-thiocytosine (**4**): Yield: 90%, mp 88—89 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, *J*=6.8 Hz, CH_3), 1.25 (24H, br s, CH_2×12), 1.61 (2H, br t, $COCH_2C\underline{H}_2$), 2.35 (2H, m, $COCH_2$), 4.16 (2H, br s, 3′-H and 4′-H), 4.33 (1H, m, 5′-H), 4.46 (1H, m, 5′-H), 4.73 (1H, br s, 2′-H), 6.12 (1H, d, *J*=7.3 Hz, 5-H), 6.91 (1H, s, 1′-H), 7.74 (1H, d, *J*=7.3 Hz, 6-H). FAB-MS *m/z*: 498 (17, MH^+), 371 (2, M^+−base), 239 (2 $C_{15}H_{31}CO^+$), 128 (100, base+$2H^+$). *Anal.* Calcd for $C_{25}H_{43}N_3O_5S$: C, 60.33; H, 8.71; N, 8.44. Found: C, 60.75; H, 9.24; N, 8.15.

1-(5′-*O*-Stearoyl-β-D-arabinofuranosyl)-2-thiocytosine (**5**): Yield: 53%, mp 120—122 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.86 (3H, t, *J*=6.8 Hz, CH_3), 1.23 (28H, br s, CH_2×14), 1.53 (2H, m, $COCH_2C\underline{H}_2$), 2.33 (2H, t, *J*=7.3 Hz, $COCH_2$), 3.96 (1H, br s, 3′-H), 4.08 (1H, ddd, *J*=2.0, 4.4, 8.3 Hz, 4′-H), 4.21 (1H, dd, *J*=4.4, 11.7 Hz, 5′-H), 4.25 (1H, dd, *J*=3.4 Hz, 2′-H), 4.38 (1H, dd, *J*=8.3, 11.7 Hz, 5′-H), 6.47 (1H, d, *J*=7.8 Hz, 5-H), 6.72 (1H, d, *J*=3.4 Hz, 1′-H), 7.89 (1H, d, *J*=7.8 Hz, 6-H). FAB-MS *m/z*: 526 (13, MH^+), 128 (100, base+$2H^+$). *Anal.* Calcd for $C_{27}H_{47}N_3O_5SHCl$: C,57.68; H,8.60; N, 7.47. Found: C, 57.32; H, 8.60; N, 7.48.

General Procedure for Obtaining 3′-*O*-Ester Derivatives (6—8) O^2,2′-Anhydro-1-(3′-*O*-acyl-β-D-arabinofuranosyl)cytosine hydrochloride

* To whom correspondence should be addressed.

Table 1. Chemical Structures of AraSC and Its Derivatives



Comp. No.	X	Y	Z	mp (°C)
AraSC	H	H	H	125—126
1	H	$CO(CH_2)_6CH_3$	H	Syrup
2	H	$CO(CH_2)_{10}CH_3$	H	111—113
3	H	$CO(CH_2)_{12}CH_3$	H	124—125
4	H	$CO(CH_2)_{14}CH_3$	H	88—89
5	H	$CO(CH_2)_{16}CH_3$	H	120—122
6	H·HCl	H	$CO(CH_2)_6CH_3$	180—181
7	H·HCl	H	$CO(CH_2)_{12}CH_3$	179—180
8	H·HCl	H	$CO(CH_2)_{16}CH_3$	176—177
9	$CO(CH_2)_7CH_3$	H	H	101—102
10	$CO(CH_2)_{12}CH_3$	H	H	105—107
11	$CO(CH_2)_{14}CH_3$	H	H	102—104
12	$CO(CH_2)_{16}CH_3$	H	H	115—116
13	$CO(CH_2)_{18}CH_3$	H	H	92—93
14	H	$PO(ONa)O(CH_2)_7CH_3$	H	195—197 (dec.)
15	H	$PO(ONa)O(CH_2)_{13}CH_3$	H	200 (dec.)
16	H	$PO(ONa)O(CH_2)_{17}CH_3$	H	214—216 (dec.)

was obtained by the reaction of 2-acyloxyisobutyryl chloride and cytidine.[3] O^2,2′-Anhydro-1-(3′-*O*-acyl-β-D-arabinofuranosyl)cytosine hydrochloride (10 mmol), NH_4HCO_3 (40 mmol) and NaSH (40 mmol) were added to anhydrous *N*,*N*-dimethylformamide (20 ml), and stirred for 15 h at room temperature. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed twice with NaCl-saturated water, and dried with Na_2SO_4. After removal of the solvent under reduced pressure, the residue was dissolved in methanol and aq. HCl was added dropwise. The deposited crystals were filtered and dried to obtain 1-(3′-*O*-acyl-β-D-arabinofuranosyl)-2-thiocytosine hydrochloride.

1-(3′-*O*-Octanoyl-β-D-arabinofuranosyl)-2-thiocytosine Hydrogen Chloride (**6**): Yield: 45%, mp 180—181 °C. ^{1}H-NMR (DMSO-d_6+D_2O) δ: 0.86 (3H, t, *J*=6.8Hz, CH_3), 1.27 (8H, br s, CH_2×4), 1.56 (2H, br t, *J*=6.8 Hz, $COCH_2C\underline{H}_2$), 2.38 (2H, t, *J*=7.3 Hz, $COCH_2$), 3.68 (2H, m, 5′-H), 4.07 (1H, m, 4′-H), 4.38 (1H, dd, *J*=1.0, 3.4 Hz, 2′-H), 4.97 (1H, s, 3′-H), 6.37 (1H, d, *J*=7.8 Hz, 5-H), 6.67 (1H, d, *J*=3.4 Hz, 1′-H), 8.00 (1H, d, *J*=7.8 Hz, 6-H). FAB-MS *m/z*: 386 (50, MH^+), 259 (3, M^+−base), 128 (100, base+2H$^+$). *Anal.* Calcd for $C_{17}H_{27}N_3O_5SHCl$: C, 48.39; H, 6.69; N, 9.96. Found: C, 47.99; H, 6.86; N, 9.82.

1-(3′-*O*-Myristoyl-β-D-arabinofuranosyl)-2-thiocytosine Hydrogen Chloride (**7**): Yield: 69%, mp 179—180 °C. ^{1}H-NMR (DMSO-d_6+D_2O) δ: 0.86 (3H, t, *J*=6.8 Hz, CH_3), 1.24 (20H, br, s, CH_2×10), 1.54 (2H, m, $COCH_2C\underline{H}_2$), 2.37 (2H, t, *J*=7.3 Hz, $COCH_2$), 3.66 (1H, dd, *J*=5.9, 11.7 Hz, 5′-H), 3.71 (1H, dd, *J*=6.4, 11.7 Hz, 5′-H), 4.06 (1H, m, 4′-H), 4.38 (1H, d, *J*=3.4 Hz, 2′-H), 4.97 (1H, s, 3′-H), 6.46 (1H, d, *J*=7.8 Hz, 5-H), 6.63 (1H, d, *J*=3.4 Hz, 1′-H), 8.03 (1H, d, *J*=7.8 Hz, 6-H). FAB-MS *m/z*: 470 (15, MH^+), 343 (3, M^+−base), 211 (3, $C_{13}H_{27}CO^+$), 128 (100, base+2H$^+$). *Anal.* Calcd for $C_{23}H_{39}N_3O_5SHCl$: C, 54.58; H, 7.97; N, 8.30. Found: C, 54.81; H, 8.14; N, 8.44.

1-(3′-*O*-Stearoyl-β-D-arabinofuranosyl)-2-thiocytosine hydrogen chloride (**8**): Yield: 50%, mp 176—177 °C. ^{1}H-NMR (DMSO-d_6+D_2O) δ: 0.86 (3H, t, *J*=6.8 Hz, CH_3), 1.24 (28H, br s, CH_2×14), 1.55 (2H, br t, *J*=6.8 Hz, $COCH_2C\underline{H}_2$), 2.37 (2H, t, *J*=7.3 Hz, $COCH_2$), 3.66 (1H, dd, *J*=5.9, 11.7 Hz, 5′-H), 3.70 (1H, dd, *J*=6.8, 11.7 Hz, 5′-H), 4.06 (1H, m, 4′-H), 4.38 (1H, d, *J*=3.4 Hz, 2′-H), 4.97 (1H, s, 3′-H), 6.46 (1H, d, *J*=7.8 Hz, 5-H), 6.63 (1H, d, *J*=3.4 Hz, 1′-H), 8.03 (1H, d, *J*=7.8 Hz, 6-H). FAB-MS *m/z*: 526 (10, MH^+), 399 (2, M^+−base), 128 (100, base+2H$^+$). *Anal.* Calcd for $C_{27}H_{47}N_3O_5SHCl$: C, 57.68; H, 8.60; N, 7.47. Found: C, 57.34; H, 8.52; N, 7.59.

General Procedure for Obtaining N^4-acyl Derivatives. (9—13) To a solution of araSC (5.0 mmol) in 25 ml pyridine was added trimethylchlorosilane (25.0 mmol) dropwise. The mixture was stirred for 30 min at room temperature, and the appropriate acyl chloride (5.5 mmol) was added to the solution, followed by stirring for 1 h. The reaction mixture was cooled in an ice bath, and water was added. The mixture was concentrated under reduced pressure, diluted with chloroform, washed twice with water, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was diluted with chloroform (30 ml) and 3.5 ml trifluoroacetic acid was added, followed by stirring for 30 min. The mixture was concentrated under reduced pressure, diluted with chloroform, washed twice with water, and dried with $MgSO_4$. The chloroform layer was concentrated *in vacuo* and the residue was crystallized from methanol to afford N^4-acyl-araSC.

N^4-Nonanoyl-1-(β-D-arabinofuranosyl)-2-thiocytosine (**9**): Yield: 53%, mp 101—102 °C. ^{1}H-NMR (DMSO-d_6+$CDCl_3$) δ: 0.88 (3H, t, *J*=6.4 Hz, CH_3), 1.28 (10H, m, CH_2×5), 1.67 (2H, m, $COCH_2C\underline{H}_2$), 2.43 (2H, t, *J*=6.8 Hz, $COCH_2$), 3.87 (2H, m, 5′-H), 4.20 (2H, m, 3′-H and 4′-H), 4.63 (1H, m, 2′-H), 5.37 (3H, br s, OH), 6.95 (1H, d, *J*=4.0 Hz, 1′-H), 7.70 (1H, d, *J*=7.6 Hz, 5-H), 8.42 (1H, d, *J*=7.6Hz, 6-H), 10.50 (1H, br s, NH). FAB-MS *m/z*: 400 (35, MH^+), 268 (100, N-(COC_8H_{17})-base+2H$^+$), 128 (80, base+2H$^+$). *Anal.* Calcd for $C_{18}H_{29}N_3O_5S$: C, 54.12; H, 7.32; N, 10.52. Found: C, 54.51; H, 7.54; N, 10.54.

N^4-Myristoyl-1-(β-D-arabinofuranosyl)-2-thiocytosine (**10**): Yield: 82%, mp 105—107 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.85(3H, t, *J*=6.4 Hz, CH_3), 1.24 (20H, m, CH_2×10), 1.55 (2H, m, $COCH_2C\underline{H}_2$), 2.41 (2H, t, *J*=6.8 Hz, $COCH_2$), 3.64 (2H, m, 5′-H), 3.93 (2H, m, 3′-H and 4′-H), 4.36 (1H, m, 2′-H), 5.09 (1H, br s, 5′-OH), 5.52 (2H, m, 2′-OH and 3′-OH), 6.87 (1H, d, *J*=3.6 Hz, 1′-H), 7.59 (1H, d, *J*=7.2 Hz, 5-H), 8.24 (1H, d, *J*=7.2 Hz, 6-H), 11.27 (1H, br s, NH). FAB-MS *m/z*: 470 (35, MH^+), 338 (100, N-$(COC1_3H_{27})$-base+2H$^+$), 128 (80, base+2H$^+$). *Anal.* Calcd for $C_{23}H_{39}N_3O_5S$: C, 58.82; H, 8.37; N, 8.95. Found: C, 58.46; H, 8.56; N, 8.80.

N^4-Palmitoyl-1-(β-D-arabinofuranosyl)-2-thiocytosine (**11**): Yield: 82%, mp 102—104 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.85 (3H, t, *J*=6.8 Hz, CH_3), 1.24 (24H, m, CH_2×12), 1.54 (2H, m, $COCH_2CH_2$), 2.40 (2H, t, *J*=7.2 Hz, $COCH_2$), 3.64 (2H, m, 5′-H), 3.93 (2H, m, 3′-H and 4′-H), 4.36 (1H, m, 2′-H), 5.09 (1H, t, *J*=5.6 Hz, 5′-OH), 5.51 (2H, m, 2′-OH and 3′-OH), 6.87 (1H, d, *J*=3.6 Hz, 1′-H), 7.59 (1H, d, *J*=7.2 Hz, 5-H), 8.24 (1H, d, *J*=7.2 Hz, 6-H), 11.26 (1H, br s, NH). FAB-MS *m/z*: 498 (21, MH^+), 366 (80, N-$(COC_{15}H_{31})$-base+2H$^+$), 128 (100, base+2H$^+$). *Anal.* Calcd for $C_{25}H_{43}N_3O_5S$: C, 60.33; H, 8.71; N, 8.44. Found: C, 59.98; H, 8.89; N, 8.34.

N^4-Stearoyl-1-(β-D-arabinofuranosyl)-2-thiocytosine (**12**): Yield: 81%, mp 115—116 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.85 (3H, t, *J*=6.8 Hz, CH_3), 1.24 (28H, m, CH_2×14), 1.54 (2H, m, $COCH_2C\underline{H}_2$), 2.41 (2H, t, *J*=7.2 Hz,

$COCH_2$), 3.64 (2H, m, 5′-H), 3.93 (2H, m, 3′-H and 4′-H), 4.36 (1H, m, 2′-H), 5.09 (1H, br s, 5′-OH), 5.52 (2H, m, 2′-OH and 3′-OH), 6.87 (1H, d, J=3.6 Hz, 1′-H), 7.59 (1H, d, J=8.0 Hz, 5-H), 8.24 (1H, d, J=8.0 Hz, 6-H), 11.27 (1H, br s, NH). FAB-MS *m/z*: 526 (15, MH^+), 394 (60, N-($COC_{17}H_{35}$)-base+$2H^+$), 128 (100, base+$2H^+$). *Anal.* Calcd for $C_{27}H_{47}N_3O_5S$: C, 61.68; H, 9.01; N, 7.99. Found: C, 61.98; H, 9.05; N, 7.81.

N^4-Eicosanoyl-1-(β-D-arabinofuranosyl)-2-thiocytosine (**13**): Yield: 70%, mp 92—93 °C. ^{1}H-NMR (DMSO-d_6+$CDCl_3$) δ: 0.88 (3H, t, J=6.4 Hz, CH_3), 1.20 (32H, m, CH_2×16), 1.63 (2H, m, $COCH_2C\underline{H}_2$), 2.44 (2H, t, J=7.6 Hz, $COCH_2$), 3.81 (2H, m, 5′-H), 4.12 (1H, m, 4′-H), 4.18 (1H, m, 3′-H), 4.47 (1H, m, 2′-H), 5.31 (3H, m, OH), 6.99 (1H, d, J=3.6 Hz, 1′-H), 7.71 (1H, d, J=7.6 Hz, 5-H), 8.42 (1H, d, J=7.6 Hz, 6-H), 10.85 (1H, br s, NH). FAB-MS *m/z*: 554 (10, MH^+), 422 (40, N-($COC_{19}H_{39}$)-base+$2H^+$), 128 (100, base+2H+). *Anal.* Calcd for $C_{29}H_{51}N_3O_5S$: C, 62.90; H, 9.28; N, 7.59. Found: C, 63.25; H, 9.34; N, 7.49.

General Procedure for Obtaining 5′-*O*-(*O*-alkylphosphate) Derivatives (14—16) A solution of araSC (5.0 mmol) and trimethylphosphate (20 ml) was cooled to −10 °C, and phosphoryl chloride (7.5 ml) was added dropwise. After stirring for 2 h at room temperature, the appropriate alcohol (20 mmol) dissolved in 40 ml tetrahydrofuran (THF) was added, followed by stirring at room temperature for 18 h. The reaction mixture was poured into ice-water (200 ml) containing $NaHCO_3$ (1.89 g). After extraction with diethyl ether, the aqueous layer was adjusted to pH 1—2, and extracted with $CHCl_3$. The $CHCl_3$ layer was concentrated under reduced pressure, and the residue was recrystallized from $CHCl_3$–EtOH to afford 5′-*O*-(*O*-alkylphosphate) derivatives. This material was suspended in 20 ml water, and adjusted to pH 7 by addition of 1 N NaOH. After addition of MeOH (100 ml) to the mixture, the solution was cooled to 4—5 °C. The insoluble materials were filtered off, and the filtrate was concentrated under reduced pressure. The residue was crystallized from H_2O–EtOH to afford 5′-*O*-(*O*-alkylphosphate) sodium salt.

1-(5′-*O*-(*O*-Octylphosphate)-β-D-arabino-furanosyl)-2-thiocytosine Sodium Salt (**14**): Yield: 25%, mp 195—197 °C (decomp.). ^{1}H-NMR (D_2O) δ: 0.72 (3H, t, J=6.8 Hz, CH_3), 1.11 (10H, m, CH_2×5), 1.50 (2H, m, $P(O)OCH_2C\underline{H}_2$), 3.77 (2H, m, 3′-H and 4′-H), 4.02—4.10 (4H, m, 5′-H and $P(O)OCH_2$), 4.55 (1H, m, 2′-H), 6.27 (1H, d, J=8.0 Hz, 5-H), 6.96 (1H, d, J=5.6 Hz, 1′-H), 7.92 (1H, d, J=8.0 Hz, 6-H). FAB-MS *m/z*: 496 (10, MNa^+), 474 (10, MH^+), 347 (12, M^+−base), 128 (100, base+$2H^+$). *Anal.* Calcd for $C_{17}H_{29}N_3O_7PSNa$: C, 43.13; H, 6.17; N, 8.88. Found: C, 42.84; H, 6.30; N, 8.61.

1-(5′-*O*-(*O*-Myristylphosphate)-β-D-arabinofuranosyl)-2-thiocytosine Sodium Salt (**15**): Yield: 54%, mp 200 °C (decomp.). ^{1}H-NMR (D_2O) δ: 0.65 (3H, t, J=6.8 Hz, CH_3), 1.03 (22H, m, CH_2×11), 1.49 (2H, m, $P(O)OCH_2C\underline{H}_2$), 3.77 (2H, m, 3′-H and 4′-H), 3.95—4.20 (4H, m, 5′-H and $P(O)OCH_2$), 4.59 (1H, m, 2′-H), 6.33 (1H, d, J=7.6 Hz, 5-H), 6.87 (1H, d, J=5.6 Hz, 1′-H), 8.00 (1H, d, J=7.6 Hz, 6-H). FAB-MS *m/z*: 580 (10, MNa^+), 558 (10, MH^+), 431 (15, M^+−base), 128 (100, base+$2H^+$). *Anal.* Calcd for $C_{23}H_{41}N_3O_7PSNa$: C, 49.54; H, 7.41; N, 7.54. Found: C, 49.22; H, 7.32; N, 7.36.

1-(5′-*O*-(*O*-Stearoylphosphate)-β-D-arabinofuranosyl)-2-thiocytosine Sodium Salt (**16**): Yield: 39%, mp 214—216 °C (decomp.). ^{1}H-NMR (D_2O) δ: 0.66 (3H, t, J=6.8 Hz, CH_3), 1.04 (30H, m, CH_2×15), 1.51 (2H, m, $P(O)OCH_2C\underline{H}_2$), 3.79 (2H, m, 3′-H and 4′-H), 3.95—4.25 (4H, m, 5′-H and $P(O)OCH_2$), 4.59 (1H, m, 2′-H), 6.33 (1H, d, J=7.6 Hz, 5-H), 6.87 (1H, d, J=5.6 Hz, 1′-H), 8.00 (1H, d, J=7.6 Hz, 6-H). FAB-MS *m/z*: 636 (10, MNa^+), 614 (10, MH^+), 487 (15, M^+−base), 128 (100, base+$2H^+$). *Anal.* Calcd for $C_{27}H_{49}N_3O_7PSNa$: C, 52.84; H,8.05; N, 6.85. Found: C, 52.65; H, 8.21; N, 6.74.

HPLC Analysis For quantitative analysis of araSC and its derivatives, the following mixtures were used as the mobile phase on a C18 reversed-phase (LiChrospher RP-18(e), 5 μm) 250×4 mm column at a flow rate of 1.0 ml/min: mixtures of water, methanol and acetic acid for the 3′ or 5′ esters; mixtures of water and methanol for the N^4-acyl derivatives; mixtures of 0.05 M phosphate buffer (pH 7.0) and acetonitrile for the acylphosphates; and a mixture of 0.02 M phosphate buffer (pH 7.0) and methanol for araSC. The wavelength of the spectrophotometer was set at 280 nm with an attenuation of 0.01 AUFS.

Measurement of Enzymatic Hydrolysis Rates Male ddY mice (25—28 g) were obtained from Tokyo Laboratory Animals (Saitama, Japan) and sacrificed to obtain blood, liver and intestine. The blood was centrifuged at 1000×***g*** for 15 min, and the resulting plasma was stored at −40 °C until use. The tissues were homogenized with pH 7.0 isotonic phosphate buffer (0.1 M) containing 0.19 M sucrose at 0 °C to give a concentration of 4.0% (w/v). Aliquots of 1 ml of the homogenates were transferred to small glass tubes and stored at −80 °C until use. No reduction in the enzyme activity of the stored samples, evaluated by monitoring the hydrolysis of **2**, **10** and **15**, was observed during the experimental period (~12 weeks).

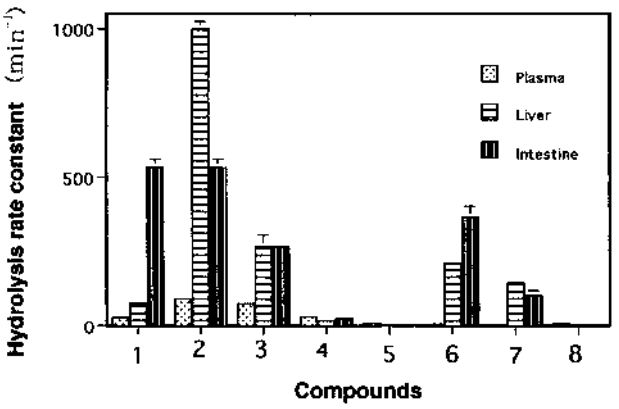


Fig. 1. Enzymatic Hydrolysis Rate of Ester Derivatives (**1**—**8**)

The enzymatic hydrolysis rates were determined in the presence of one of the enzyme systems diluted with an isotonic phosphate buffer (pH 7.0) containing 0.19 M sucrose and the experiments were perfomed at 37 °C. Hydrolysis was initiated by adding the stock solution to the test solution at an initial concentration of 4×10^{-5} M, the lowest concentration suitable for quantitative analysis of the derivatives. To confirm complete dissolution of the compound, another measurement was performed at a concentration of 8×10^{-5} M for all compounds. Changes in the concentration of both the derivatives and parent compound, araSC, were followed by HPLC analysis of samples taken periodically from the reaction mixture. The effect on enzymatic hydrolysis was evaluated as *pseudo*-first-order rate constants for 1.0% (w/v) plasma or homogenates.

Measurement of Chemical Hydrolysis Rates The chemical hydrolysis rates of the araSC derivatives were measured in an isotonic phosphate buffer (pH 7.0) containing 0.19 M sucrose at 37 °C. The reactivity was evaluated as described above.

Evaluation of Antitumor Activity Male CDF1 mice were purchased from Tokyo Laboratory Animals (Saitama, Japan). Mice (20—23 g) in groups of five were inoculated intraperitoneally with 1×10^6 P388 leukemia cells. The compound suspension was given intraperitoneally on days 1, 2, 3, 4 and 5 starting 24 h after inoculation. The antitumor activities were recorded as T/C (%), the ratio of the mean survival time of the treated group (T) to that of the control group (C). To evaluate side-effects, the body weight of animals in each group was measured at the start of the experiment and on day 5.

Results and Discussion

The enzymatic reactivity of the carboxyester derivatives (**1**—**8**) is shown in Fig. 1. These derivatives regenerate parent compound, araSC, quantitatively. Higher reactivity was observed with the C6—C10 esters, and this decreased with increasing alkyl chain length in both the 3′- and 5′-esters. The reactivity to plasma enzyme system was weaker in the 3′- than in the 5′-esters. This was similar to our previous observations on 5-fluoro-2′-deoxyuridine derivatives.[4] The enzymatic reactivities of the N^4-amides and 5′-phosphodiesters are quite different from those of the carboxyesters (Fig. 2). Reactivities of these derivatives are much weaker than those of carboxyester derivatives (note the units of the vertical axis). Reactivity to intestinal homogenate was almost equivalent, although those to plasma and liver were depended on the chemical structure. Derivatives with the shortest alkyl chain, **9** and **14**, showed higher reactivity, and this reactivity decreased with increasing alkyl chain length. Similar observations have been reported for the enzymatic regeneration of araC from its *N*4-amide prodrug, enocitabine,[5,6] and the 5′-phosphodiester prodrug, cytarabine ocfosfate.[7,8]

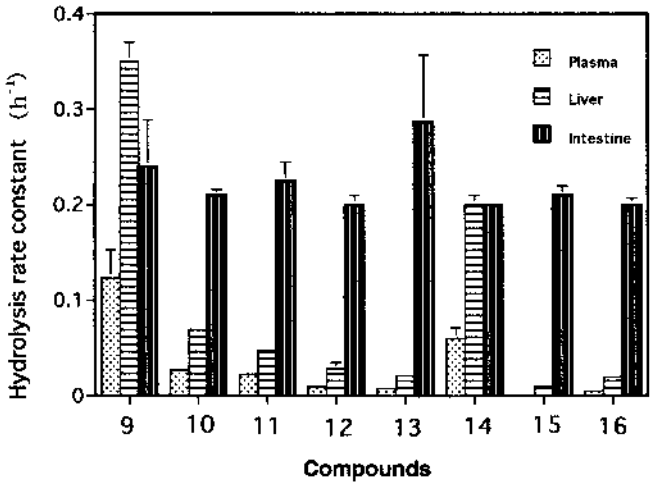


Fig. 2. Enzymatic Hydrolysis Rate of N^4-Amide and 5′-Phosphodiester Derivatives (**9**—**16**)

Table 2. Antitumor Activity of Compounds **1**—**8**

Compounds	T/C (dose[a], mg/kg/day)				Weight change[b]			
	75	100	150	300	75	100	150	300
1	—	104	—	44	—	−0.4	—	0/5[c]
2	—	100	—	50	—	−0.3	—	0/5[c]
3	—	63	—	220	—	−2.7	—	−5.0
4	—	115	—	130	—	−0.5	—	−1.5
5	134	—	155	173	+2.1	—	+1.9	−0.3
6	98	—	78	39	−0.1	—	−0.7	−3.2
7	—	113	—	124	—	+1.6	—	−0.4
8	136	—	148	138	+1.1	—	+2.3	+2.8
AraSC	—	95	—	103	—	+2.8	—	+2.5

a) Suspension was given intraperitoneally on days 1, 2, 3, 4 and 5 starting at 24 h after inoculation. *b*) Body weight was measured at the start and on day 5. Changes are indicated as an average. *c*) None of the 5 mice survived to day 5.

The antitumor activity of the carboxyester derivatives were shown in Table 2. Only compounds **3** and **5** showed activity over 150% in T/C. Compound **3** showed activity over 200% in T/C at the highest dose, although its toxicity, indicated by weight loss, was serious (5.0 g/mouse). There was no clear relationship between the antitumor activity and enzymatic reactivity of these ester derivatives. Physicochemical properties other than biological reactivity may be more important factors for their activation to parent drug.

With the exception of compound **9**, the *N*4-amide derivatives showed an antitumor effect (>150% in T/C) (Table 3). Compounds **10** and **11** showed high activity (>200% in T/C), although both resulted in marked weight loss (>5.0 g/mouse) at a dose of 300 mg/kg/d. Compound **9** showed no antitumor activity but toxic. This compound also showed relatively high reactivity to plasma and liver, as did in compound **14** which also showed little antitumor activity. Of the 5′-phosphodiesters, compounds **15** and **16** showed high activity (>200% in T/C) with very little weight loss (less than 0.5 g/mouse). All compounds had a greater reactivity to intestinal homogenate than to plasma and liver homogenate (except **9** to liver), although this high reactivity to the intestine may not reflect their activity because of the administration route used in this experiment (Table 3).

Table 3. Antitumor Activity of Compounds **9**—**16**

Compounds	T/C (dose[a], mg/kg/day)			Weight change[b]		
	75	150	300	75	150	300
9	98	94	69	+0.8	−0.6	−1.7
10	143	165	222	−2.3	−3.6	−5.5
11	143	166	223	−1.1	−4.0	−5.9
12	153	179	196	+3.0	+1.7	+2.3
13	135	150	178	+1.6	+2.2	+0.2
14	111	109	113	+5.5	+3.5	+3.8
15	105	150	189	+5.0	+1.8	−0.3
16	118	142	210	+6.1	+2.9	−0.1

a) Suspension was given intraperitoneally on days 1, 2, 3, 4 and 5 starting at 24 h after inoculation. *b*) Body weight was measured at the start and on day 5. Changes are indicated as an average.

The antitumor activity of antimetabolites is highly dependent on their release or administration rate in the body. To obtain an appropriate rate of administration, rate of enzymatic regeneration of the parent compound is manipulated by chemical modification(s). Of the derivatives studied here, compounds **15** and **16** showed promising characteristics. Both compounds showed very low reactivity to plasma and liver enzyme systems and, therefore, their regeneration of araSC is slow and prolonged release of araSC is expected. Since intraperitoneal administration was employed in this study, the intestinal enzyme system may not play a major role in the activation process. Further investigation using other administration routes (*e.g.* oral) should provide more information.

REFERENCES

1) Ruyle W. V., Shen T. Y., *J. Med. Chem.*, **10**, 331—334 (1967).
2) Kawaguchi T., Ichikawa T., Hasegawa T., Saneyoshi M., Yukita A., Asano M., Wakayama T., Kato H., Nagata T., *Biol. Pharm. Bull.*, **22**, 100—102 (1999).
3) Hamamura E. K., Prystasz M., Verheyden J. P. H., Moffatt J. G., *J. Med. Chem.*, **19**, 654—662 (1976).
4) Kawaguchi T., Fukushima S., Hayashi Y., Nakano M., *Pharm Res.*, **5**, 741—744 (1988).
5) Tsuruo T., Iida H., Hori K., Tsukagoshi S., Sakurai Y., *Cancer Res.*, **41**, 4484—4488 (1981).
6) Oh-ishi J., Kataoka T., Tsukagoshi S., Sakurai Y., Shibukawa M., Kobayashi H., *Cancer Res.*, **41**, 2501—2506 (1981).
7) Saneyoshi M., Morozumi M., Komada K., Machida H., Kuninaka A., Yoshino H., *Chem Pharm. Bull.*, **28**, 2915—2923 (1980).
8) Komada K., Morozumi M., Saito K., *Jpn. J. Cancer Res.*, **80**, 679—685 (1989).