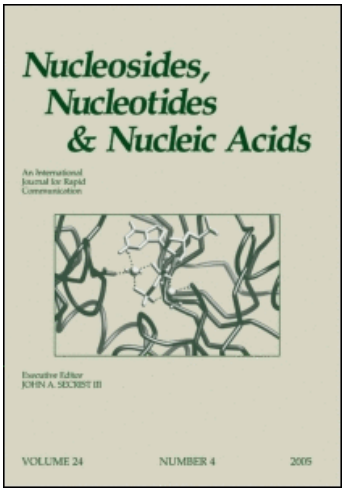


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis and Structure Activity Relationships of 5-Substituted - 4'-thio-β-D-Arabinofuranosylcytosines

Kamal N. Tiwari[a]; Anita T. Shortnacy-fowler[a]; Loredana Cappellacci[a]; William R. Waud[a]; William B. Parker[a]; John A. Montgomery[a]; John A. Secrist III[a]
[a] Southern Research Institute, Birmingham, Alabama

To cite this Article Tiwari, Kamal N. , Shortnacy-fowler, Anita T. , Cappellacci, Loredana , Waud, William R. , Parker, William B. , Montgomery, John A. and Secrist III, John A.(2000) 'Synthesis and Structure Activity Relationships of 5-Substituted - 4'-thio-β-D-Arabinofuranosylcytosines', Nucleosides, Nucleotides and Nucleic Acids, 19: 10, 2005 — 2017
To link to this Article: DOI: 10.1080/15257770008045474
URL: http://dx.doi.org/10.1080/15257770008045474

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 19(10–12), 2005–2017 (2000)

SYNTHESIS AND STRUCTURE ACTIVITY RELATIONSHIPS OF 5-SUBSTITUTED - 4′-THIO-β-D-ARABINOFURANOSYLCYTOSINES†

Kamal N. Tiwari, Anita T. Shortnacy-Fowler, Loredana Cappellacci, William R. Waud, William B. Parker, John A. Montgomery, and John A. Secrist III*
Southern Research Institute, P. O. Box 55305, Birmingham, Alabama 35255-5305

ABSTRACT: Four 5-substituted (chloro, fluoro, bromo, methyl) 1-(4-thio-β-D-arabinofuranosyl)cytosines and their α anomers were synthesized by a facile route in high yields. All of these nucleosides were evaluated for cytotoxicity against a panel of human tumor cell lines *in vitro*. Only 5-fluoro-1-(4-thio-β-D-arabinofuranosyl)cytosine was found to be highly cytotoxic in all the cell lines and was further evaluated *in vivo*.

INTRODUCTION

A number of 2′-deoxyribo and arabino nucleoside analogs have shown promise as anticancer agents.[1] The activity of 1-β-D-arabinofuranosylcytosine (ara-C, cytarabine), which is particularly useful against acute myelogenous leukemia, was the first to be observed in animals and in man. As a part of our ongoing program on the synthesis and biologic evaluation of 4′-thionucleosides,[2-7] we have reported the synthesis and antitumor activity of 1-(4-thio-β-D-arabinofuranosylcytosine, 4′-thio-ara-C).[8] Preliminary results on the mechanism of action of 4′-thio-ara-C suggest that it is metabolized to its triphosphate, which inhibits DNA polymerase and is also incorporated into DNA. 4′-Thio-ara-C appears to be a very poor substrate of cytidine deaminase as compared to that of Ara-C. This enzyme converts both compounds to the corresponding uracil nucleosides, which have little or no cytotoxicity. To date no 5-substituted analogs of 4′-thio-ara-C have been reported. In order to further explore this structure activity relationship, we undertook the

†Dedicated to the memory of Dr. Sasha Krayevsky.

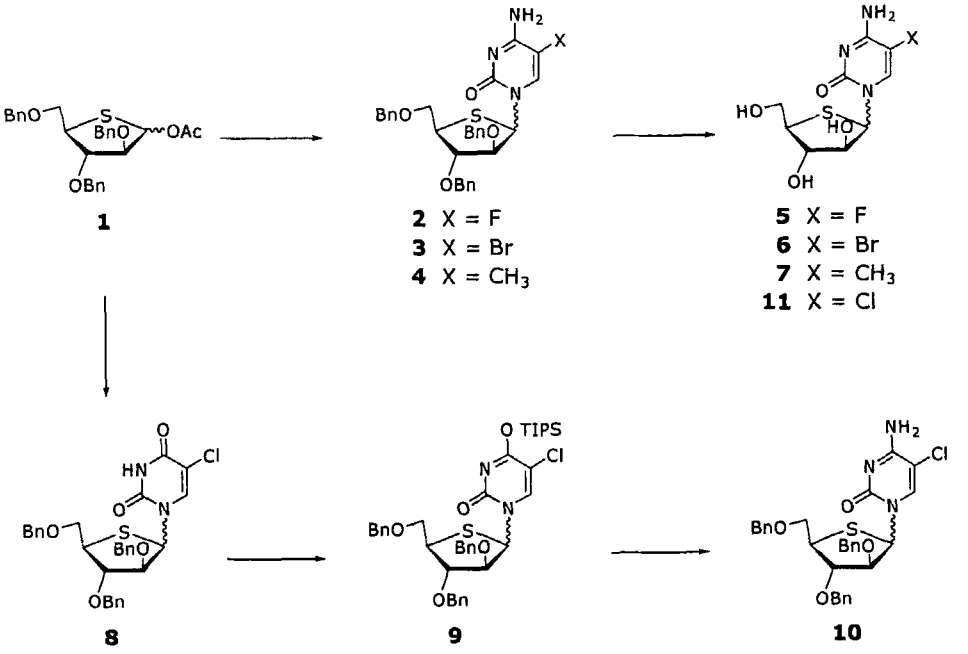
synthesis of various 5-substituted-4′-thio-ara-C compounds. The details of the synthesis and initial antitumor activity of this series of compounds are reported herein.

CHEMISTRY

We have already reported the details of a convenient synthesis of a 4-thioarabinofuranose intermediate and its conversion to a series of purine nucleoside analogs and a cytosine analog.[7,8] Coupling of sugar **1** with silylated 5-fluorocytosine, 5-bromocytosine and 5-methylcytosine *in situ* using trimethylsilyltrifluoromethane sulfonate gave a 2:1 α,β mixture of **2** (80%), **3** (75%) and **4** (76%) respectively. Chromotographic separation of these anomers was found to be very difficult in several different solvent systems, so the anomeric mixtures were deblocked using BCl_3 to afford compounds **5**, **6**, and **7** respectively. Selective crystallization of the α,β mixture of **5** in water gave pure **5β** (16%) and **5α** anomer (36%). Nucleosides **6** and **7** resolved nicely on TLC plates and were separated chromatographically to afford **6β** (23%), **6α** (43%) and **7β** (21%), **7α** (44%) respectively. 5-Chlorocytosine was not commercially available and several attempts to convert 1-(4-thio-β-D-arabinofuranosyl)uracil to the corresponding 5-chloro analog using a literature procedure (NCS and glacial acetic acid[13] at 80°C) were not successful because of sulfur oxidation. The commercial availability of 5-chlorouracil prompted us to couple it with the thiosugar **1** to obtain nucleoside **8** (58%) as 2:1 α:β mixture. The 5-chlorouracil nucleosides **8** were converted to 5-chlorocytosine nucleosides **10β** (21%) and **10α** (33%) via the 2,4,6-triisopropylbenzenesulfonate derivatives **9β** and **9α** using the standard procedure.[8] The 5-chlorocytosine nucleosides **10β** and **10α** were deblocked as described above using BCl_3 to give 5-chloro-4′-thio-ara-C (**11β**, 56%) and its α-anomer (**11α**, 62%) respectively. The anomeric configuration and point of attachment of sugar and pyrimidine in all the nucleosides were confirmed by ^1H NMR spectra using decoupling experiments and by comparison with 4′-thio-ara-C.

BIOLOGICAL DATA

The cytotoxicities of all of the new 5-substituted 4′-thio-ara-C analogs were determined against seven human cell lines,[9,10,11] identified in Table 1. Only the 5-fluoro analog **5β** was cytotoxic to all of these cell lines. The other agents were not cytotoxic at



TIPS = 2,4,6-Triisopropylbenzene sulfonate

Scheme 1

Table 1
Cytotoxicity of 4′-Thio-ara-C, 5-F-4′-Thio-Ara-C and Ara-C in Seven Human Tumor Cell Lines

Cell Line	IC_{50}[a] (μM)		
	4′-Thio-Ara-C[b]	Ara-C[b]	5F-4′-Thio-Ara-C
CAKI-1 renal carcinoma	2.2	2.5	27
SNB-7 CNS tumor	1.8	1.1	7.2
NCI-H23 nonsmall cell lung adenocarcinoma	3.6	0.59	1.8
DLD-1 colon adenocarcinoma	39	>5	141
LOX IMVI amelanotic melanoma	13	2.0	4.0
ZR-75-1 breast carcinoma	2.5	0.80	0.96
PC-3 prostate adenocarcinoma	5.8	>5	3.6

[a] IC_{50} is defined as the concentration of drug inhibiting the growth of cells after 72 h to one-half that observed in the absence of drug. The values listed are the mean of two or more determinations.
[b] See Reference 8.

the highest concentration utilized (100 μM). The 5-fluoro analog **5β** was comparable in cytotoxicity to the parent compound 4′-thio-ara-C, showing greater toxicity in about half the cell lines, and lesser toxicity in the others. Based upon this data, **5β** was selected for evaluation in animal models. Our initial experiment was conducted in the CAKI 1 renal human tumor xenograft model in mice (Table 2). In this tumor, treatment with **5β** resulted in one cure as well as a significant delay in tumor growth for the other mice. These results are comparable to those seen in the same xenograft model for 4′-thio-ara-C[8], where cures and delays in tumor growth were also seen.[12]

EXPERIMENTAL SECTION

Melting points were determined on a Mel-Temp apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Nicolet NT-300 NB spectrometer operating at 300.635 MHz (^{1}H) at 22 °C. Chemical shifts are expressed in parts per million downfield from tetramethylsilane. Anomeric assignments were based upon chemical shifts and coupling constants, including comparisons with the parent compound, 4′-thio-ara-C, for which we have carried out standard NOE experiments. These experiments, carried out in D2O, demonstrated for the β anomer a 4.9% enhancement of the signal for H-3′ upon irradiation of H-6. The corresponding α anomer showed 3.2% and 4.4% enhancements for H-1′and H-3′ upon irradiation of the other proton of this pair. The chemical shifts and coupling constants for the compounds herein were consistent with those of the α and β anomers of the parent compound 4′-thio-ara-C. UV absorption spectra were determined with a Perkin-Elmer Lambda 9 spectrometer by dissolving each compound in methanol or water and diluting 10-fold with 0.1 N HC1, pH 7 buffer, or 0.1 N NaOH. Numbers in parentheses are extinction coefficients (x 10^{-3}), sh = shoulder. Microanalyses were performed by the Spectroscopic and Analytical Laboratory of Southern Research Institute. Mass spectra were recorded on a Varian/MAT 311A double-focusing mass spectrometer in the fast atom bombardment (FAB) mode. HPLC analyses were carried out on a Hewlett-Packard 1100 series liquid chromatograph with a Phenomenex Sphereclone C_{18} column (4.6 mm x 250 mm) and UV monitoring (254 nm). Flash chromatographic separations were carried out by using 230-400 mesh silica gel from E. Merck. TLC was carried out on Analtech precoated (250 μm) silica gel (GF) plates. Ion

Table 2
Response of SC CAKI-1 Renal Tumor to Treatment

Treatment					Tumor Regression				
Agent	Dosage (Mg/Kg/ Dose)	Rt	Schedule	Non-Specific Deaths/ Total	Number of Partial	Number of Complete	Tumor Free Surv/ Total	Days to 3 Doubling	Days Delay (T-C)
Control		IP	Days 12-20				0/12	11.3	
5-fluoro-1-(4-thio-β-D-arabinofuransyl)cytosine	30	IP	Days 12-20	2/6	0	4	1/6	>55.0	>43.8
5-fluoro-1-(4-thio-β-D-arabinofuransyl)cytosine	20	IP	Days 12-20	0/6	0	6	1/6	>55.0	>43.8
5-fluoro-1-(4-thio-β-D-arabinofuransyl)cytosine	13.3	IP	Days 12-20	0/6	0	6	1/6	54.0	42.7

NCr-nu athymic mice were implanted sc with tumor fragments. The day of implantation was designated Day 0. 5-Fluoro-4'-thio-ara-C was tested at several dosage levels. Treatment began when the median tumor size was approximately 170 mg. Antitumor activity was assessed on the basis of delay in tumor growth (T-C) which is the difference in the median of times poststaging for tumors of the treated (T) and control (C) groups to double in mass three times. Drug deaths and any animal that died whose tumor failed to grow to the evaluation size were excluded.

exchange chromatography was done on Dowex 50W-X8, 100-200 mesh ion exchange resin.

Cell Culture Cytotoxicity Data. All cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum, sodium bicarbonate, and 2 mM L-glutamine. For *in vitro* evaluation cells were plated in 96-well microtiter plates and then were exposed continuously to various concentrations of the compounds for 72 h at 37°C. Cell viability was measured using either the neutral red assay (absorbance read at 550 nm) or the sulforhodamine B assay (absorbance read at 570 nm). The background absorbance mean was subtracted from the data followed by conversion to percent of control. The drug concentrations producing survival just above and below the 50% level were used in a linear regression analysis to calculate the IC_{50}.

5-Fluoro-1-(2,3,5-tri-*O*-benzyl-4-thio-α,β-D-arabinofuranosyl)cytosine (2). To a suspension of 1-*O*-acetyl-2,3,5-tri-*O*-benzyl-4-thio-D-arabinofuranose (478 mg, 1 mmol) and 5-fluorocytosine (161.0 mg, 1.25 mmol) in anhydrous acetonitrile (25 ml) were added consecutively hexamethyldisilazane (HMDS, 162 mg, 1.0 mmol) and chlorotrimethylsilane (TMSCl, 434 mg, 4.0 mmol). The mixture was stirred at room temperature for 0.5 h then cooled to -78 °C. Trimethylsilyltrifluoromethane sulfonate (267 mg, 1.2 mmol) was added, and the resulting solution was stirred at -78 °C for another 2.5 h. The mixture was warmed to room temperature, concentrated to a small volume (5 mL), diluted with CH_2Cl_2 (50 mL) and then washed with water (20 mL) followed by saturated sodium bicarbonate and water. The organic layer was dried over $MgSO_4$ and evaporated to dryness. The residue was purified by silica gel chromatography (50 g. elution with $CHCl_3$/MeOH 98:2) to afford **2** (436 mg, 80%) as a colorless syrup; TLC (95:5 $CHCl_3$/MeOH) R_f 0.65; as 2:1 α,β mixture (HPLC); MS z/e 553 $(M+Li)^+$.

^1H NMR ($CDCl_3$) δ 8.40 (d, 1H, H-6_β, J = 7.6); 8.10 (d, 1H, H-6_α, $J_{5,6}$ = 7.5 Hz); 7.38-7.09 (m, 30H, aromatic H's); 6.65 (dd, 1H, H-$1'_\beta$, $J_{1',5\text{-F}}$ = 1.9 Hz, $J_{1',2'}$ = 5.2 Hz); 6.25 (t, 1H, H-$1'_\alpha$, $J_{1',2'}$ = 1.6 Hz, $J_{1',5\text{-F}}$ = 1.6 Hz); 4.30-4.95 (overlapping multiplets, 12H, $C_6H_5CH_2$); 4.16-4.22 (m, 4H H-$2'_{\alpha,\beta}$, H-$3'_{\alpha,\beta}$); 3.90-3.94 (m, 1H, H-$4'_\alpha$); 3.74-3.82 (m, 1H, H-$5'_\alpha$); 3.60-3.70 (m, 2H, H-$5'_\beta$); 3.52-3.56 (m, 1H, H-$5'_\alpha$); 3.40-3.46 (m, 1H, H-$4'_\beta$).

5-Fluoro-1-(4-thio-β-D-arabinofuranosyl)cytosine (5β) and α anomer (5α). To a 1 M solution of boron trichloride in dry CH_2Cl_2 (7 mL, 7 mmol) cooled to -78 °C was added dropwise over a period of 30 min, a solution of compound **2** (273 mg, 0.5 mmol) in dry CH_2Cl_2 (10 mL). The reaction mixture was stored overnight at -20 °C. The solvent was removed in vacuo and the residue was coevaporated with CH_2Cl_2 (4 x 20 mL). The residue was neutralized with saturated $NaHCO_3$ (25 mL) and the solution was extracted with chloroform (15 mL). The aqueous layer was applied to a cation exchange (H^+) column that was eluted with water to remove salts. Further elution with 1N NH_4OH provided the desired compound **5** (113 mg, 82%) as 2:1 α,β mixture (HPLC) which upon crystallization with methanol gave **5α** (50 mg, 36%), m.p. 219 °C; MS z/e 278 $(M+H)^+$. The mother liquor was evaporated to a residue which was crystallized from water to obtain **5β** (22 mg, 16%), m.p. 209 °C; MS z/e 278 $(M+H)^+$.

5β ^{1}H NMR (Me_2SO-d_6) δ 8.16 (d, 1H, H-6, $J_{6,5\text{-F}}$ = 7.6 Hz); 7.72 (bs, 1H, NH); 7.50 (bs, 1H, NH); 6.23 (dd, 1H, H-1', $J_{1',2'}$ = 4.6 Hz, $J_{1',5\text{-F}}$ = 2.1 Hz); 5.66 (bd, 1H, 2'-OH, $J_{2',2'\text{-OH}}$ = 4.0 Hz); 5.39 (d, 1H, 3'-OH, $J_{3',3'\text{-OH}}$ = 4.2 Hz); 5.20 (bt, 1H, 5'-OH, $J_{5'a,5'\text{-OH}}$ = $J_{5'b,5'\text{-OH}}$ = 4.7 Hz); 4.02-3.94 (m, 2H, H-2', H-3'); 3.74 (dd, 1H, H-5'b, $J_{4',5'b}$ = 5.2 Hz, $J_{5'a,5'b}$ = 11.1 Hz, $J_{5'b,5'\text{-OH}}$ = 4.7 Hz); 3.64 (ddd, 1H, H-5'a, $J_{4',5'a}$ = 6.1 Hz, $J_{5'a,5'b}$ = 11.1 Hz, $J_{5'a,5'\text{-OH}}$ = 4.7 Hz); 3.17 (ddd, 1H, H-4', $J_{3',4'}$ = 4.1 Hz, $J_{4',5'a}$ = 6.1 Hz, $J_{4',5'b}$ = 5.2 Hz). Anal. calcd. for $C_9H_{12}FN_3O_4S{\bullet}H_2O$: C, 36.60; H, 4.78; N, 14.23. Found: C, 36.79; H, 4.39; N, 14.26.

5α ^{1}H NMR (Me_2SO-d_6) δ 8.24 (d, 1H, H-6, $J_{6,5\text{-F}}$ = 7.5 Hz); 7.77 (bs, 1H, NH); 7.53 (bs, 1H, NH); 5.82 (dd, 1H, H-1', $J_{1',2'}$ = 6.8 Hz, $J_{1',5\text{-F}}$ = 2.1 Hz); 5.64 (d, 1H, 2'-OH, $J_{2',2'\text{-OH}}$ = 5.8Hz); 5.51 (d, 1H, 3'-OH, $J_{3',3'\text{-OH}}$ = 4.6 Hz); 4.91 (t, 1H, 5'-OH, $J_{5'a,5'\text{-OH}}$ = 5.0, $J_{5'b,5'\text{-OH}}$ = 5.0 Hz); 3.99 (ddd, 1H, H-2', $J_{1',2'}$ = 6.8 Hz, $J_{2',3'}$ = 7.2 Hz, $J_{2',2'\text{-OH}}$ = 5.8 Hz); 3.82 (ddd, 1H, H-5'b, $J_{4',5'b}$ = 4.2 Hz, $J_{5'a,5'b}$ = 10.6 Hz, $J_{5'b,5'\text{-OH}}$ = 5.0 Hz); 3.67 (dd, 1H, H-3', $J_{2',3'}$ = 7.2 Hz, $J_{3',3'\text{-OH}}$ = 4.6 Hz, $J_{3',4'}$ = 7.5 Hz); 3.52 (ddd, 1H, H-4', $J_{3',4'}$ = 7.5 Hz, $J_{4',5'b}$ = 4.2 Hz, $J_{4',5'a}$ = 7.9 Hz); 3.37 (ddd, 1H, H-5a', $J_{4',5'a}$ = 7.9 Hz, $J_{5'a,5'b}$ = 10.6 Hz, $J_{5'a,5'\text{-OH}}$ = 5.0 Hz). Anal. calcd. for $C_9H_{12}FN_3O_4S{\bullet}H_2O$: C, 36.60; H, 4.78; N, 14.23. Found: C, 36.82; H, 4.44; N, 14.37.

5-Bromo-1-(2,3,5-tri-*O*-benzyl-4-thio-α,β-D-arabinofuranosyl)cytosine (3). This compound was prepared at the same scale by the same procedure used for **2**, substituting

5-bromocytosine (237.5 mg, 1.25 mmol). The chromatographic purification afforded **3** (455 mg, 75%) as a colorless syrup; TLC (96:4 $CHCl_3$/MeOH) R_f 0.55; as 2:1 α,β mixture (HPLC); MS z/e 614 $(M+Li)^+$.

^1H NMR ($CDCl_3$) δ 8.70 (s, 1H, H-6_β); 8.26 (s, 1H, H-6_α); 7.10-7.40 (m, 30H, aromatic H's); 6.58 (d, 1H, H-$1'_\beta$, $J_{1',2'}$ = 5.3 Hz); 6.32 (dd, 1H, H-$1'_\alpha$, $J_{1',2'}$ = 1.3 Hz, $J_{1',3'}$ = 0.6 Hz); 4.30-4.98 (overlapping multiplets, 12H, $C_6H_5CH_2$); 4.14-4.22 (m, 4H, H-$2'_{\beta,\alpha}$ and H-$3'_{\beta,\alpha}$); 3.97 (t, 1H, H-$4'_\alpha$); 3.52-3.82 (m, 4H, H-$5'_{\alpha,\beta}$); 3.43 (m, 1H, H-$4'_\beta$).

5-Bromo-1-(4-thio-β-D-arabinofuranosyl)cytosine (6β) and α anomer (6α). The same procedure that was presented for the **5α/5β** mixture was employed, starting with 303 mg (0.5 mmol) of **3.** The cation exchange column provided the desired compound **6** (140 mg, 83%) as 2:1 α,β mixture (HPLC) which was separated by preparative TLC ($CHCl_3$:MeOH 3:1) to give **6α** (72 mg, 43%), m.p. 189 °C (EtOH); MS z/e 338 $(M+H)^+$ and **6β** (39 mg, 23%), m.p. 183 °C (H_2O); MS z/e 338 $(M+H)^+$.

6β ^1H NMR (Me_2SO-d_6) δ 8.32 (s, 1H, H-6); 7.84 (bs, 1H, NH); 7.00 (bs, 1H, NH); 6.26 (d, 1H, H-1', $J_{1',2'}$ = 4.6 Hz); 5.72 (d, 1H, 2'-OH, $J_{2',2'\text{-OH}}$ = 5.1Hz); 5.42 (d, 1H, 3'-OH, $J_{3',3'\text{-OH}}$ = 4.0 Hz); 5.25 (t, 1H, 5'-OH, $J_{5'a,5'\text{-OH}}$ = $J_{5'b,5'\text{-OH}}$ = 5.0 Hz); 4.00-3.94 (m, 2H, H-2', H-3'); 3.73 (dd, 1H, H-5'b, $J_{4',5'b}$ = 5.2 Hz, $J_{5'a,5'b}$ = 11.1 Hz, $J_{5'b,5'\text{-OH}}$ = 8.1 Hz); 3.64 (ddd, 1H, H-5'a, $J_{4',5'a}$ = 6.1 Hz, $J_{5'a,5'b}$ = 11.1 Hz, $J_{5'a,5'\text{-OH}}$ = 5.0 Hz); 3.18 (ddd, 1H, H-4', $J_{3',4'}$ = 4.1 Hz, $J_{4',5'a}$ = 6.1 Hz, $J_{4',5'b}$ = 5.2 Hz). Anal. calcd. for $C_9H_{12}BrN_3O_4S$•0.2 H_2O: C, 31.71; H, 3.66; N, 12.33. Found: C, 31.55; H, 3.53; N, 12.16.

6α ^1H NMR (Me_2SO-d_6) δ 8.33 (s, 1H, H-6); 7.78 (bs, 1H, NH); 7.04 (bs, 1H, NH); 5.81 (d, 1H, H-1', $J_{1',2'}$ = 6.4 Hz); 5.67 (d, 1H, 2'-OH, $J_{2',2'\text{-OH}}$ = 5.7Hz); 5.48 (d, 1H, 3'-OH, $J_{3',3'\text{-OH}}$ = 4.6 Hz); 5.25 (t, 1H, 5'-OH, $J_{5'a,5'\text{-OH}}$ = 6.0 Hz, $J_{5'b,5'\text{-OH}}$ = 4.6 Hz); 4.07 (ddd, 1H, H-2', $J_{1',2'}$ = 6.4 Hz, $J_{2',3'}$ = 6.8 Hz, $J_{2',2'\text{-OH}}$ = 5.7 Hz); 3.81 (ddd, 1H, H-5'b, $J_{4',5'b}$ = 4.2 Hz, $J_{5'a,5'b}$ = 10.7 Hz, $J_{5'b,5'\text{-OH}}$ = 4.6 Hz); 3.69 (ddd, 1H, H-3', $J_{2',3'}$ = 6.8 Hz, $J_{3',3'\text{-OH}}$ = 4.6 Hz, $J_{3',4'}$ = 6.8 Hz); 3.57 (ddd, 1H, H-4', $J_{3',4'}$ = 6.8 Hz, $J_{4',5'b}$ = 4.2 Hz, $J_{4',5'a}$ = 8.2 Hz); 3.38 (ddd, 1H, H-5'a, $J_{4',5'a}$ = 8.2 Hz, $J_{5'a,5'b}$ = 10.7 Hz, $J_{5'a,5'\text{-OH}}$ = 6.0 Hz). Anal. calcd. for $C_9H_{12}BrN_3O_4S$•0.2 H_2O: C, 31.71; H, 3.66; N, 12.33. Found: C, 31.64; H, 3.54; N, 12.13.

5-Methyl-1-(2,3,5-tri-*O*-benzyl-4-thio-α,β-D-arabinofuranosyl)cytosine (4). This compound was prepared at the same scale by the same procedure used for **2**, substituting 5-methylcytosine (156 mg, 1.25 mmol). The chromatographic purification afforded **4** (412 mg, 76%) as a colorless syrup; TLC (96:4 $CHCl_3$/MeOH) R_f 0.55; as 2:1 α,β mixture (HPLC); MS z/e 550 $(M+Li)^+$.

^1H NMR ($CDCl_3$) δ 8.02 (bd, 1H, H-6_β, J = 1.6); 7.78 (d, 1H, H-6_α, J = 1.6 Hz); 7.18-7.38 (m, 30H, aromatic H's); 6.68 (d, 1H, H-1'$_\beta$, $J_{1',2'}$ = 5.1 Hz); 6.40 (d, 1H, H-1'$_\alpha$, $J_{1',2'}$ = 1.3 Hz); 4.34-5.0 (overlapping multiplets, 12H, $C_6H_5CH_2$); 4.16-4-32 (m, 4H, H-2'$_{\beta,\alpha}$, H-3'$_{\beta,\alpha}$); 3.95 (m, 1H, H-4'$_\alpha$,); 3.72 -3.82 (m, 2H, H-5'$_\alpha$, H-5'$_\beta$); 3.54-3.68 (m, 2H, H-5'$_\alpha$, H-5'$_\beta$); 3.45 (m, 1H, H-4'$_\beta$); 1.64 (d, 3H, $CH_{3\alpha}$, J = 1.6Hz); 1.60 (d, 3H, $CH_{3\beta}$, J = 1.6Hz).

5-Methyl-1-(4-thio-β-D-arabinofuranosyl)cytosine (7β) and α anomer (7α). The same procedure that was presented for the **5α/5β** mixture was employed, starting with 272 mg (0.5 mmol) of **4**. The cation exchange column provided the desired compound **7** (106 mg, 78%) as 2:1 α,β mixture (HPLC) which was separated by preparative TLC ($CHCl_3$:MeOH 3:1) to give **7α** (60 mg, 44%), m.p. 221 °C (EtOH), MS z/e 274 $(M+H)^+$ and **7β** (29 mg, 21%), m.p. 210 °C (H_2O); MS z/e 274 $(M+H)^+$.

7β ^1H NMR (Me_2SO-d_6) δ 7.80 (q, 1H, H-6, $J_{5\text{-}CH3,6}$ = 0.8 Hz); 7.23 (bs, 1H, NH); 6.80 (bs, 1H, NH); 6.30 (d, 1H, H-1', $J_{1',2'}$ = 5.1 Hz); 5.50-5.25 (bs, 3H, 2'-OH, 3'-OH, 5'-OH); 3.98 (dd, 1H, H-2', $J_{1',2'}$ = 5.1 Hz, $J_{2',3'}$ = 5.5 Hz); 3.93 (dd, 1H, H-3', $J_{2',3'}$ = 5.5 Hz, $J_{3',4'}$ = 5.5 Hz); 3.77 (dd, 1H, H-5'b, $J_{4',5'b}$ = 5.3 Hz, $J_{5'a,5'b}$ = 11.0 Hz); 3.64 (dd, 1H, H-5'a, $J_{4',5'a}$ = 6.4 Hz, $J_{5'a,5'b}$ = 11.0 Hz); 3.36 (ddd, 1H, H-4', $J_{3',4'}$ = 6.4 Hz, $J_{4',5'a}$ = 5.5 Hz, $J_{4',5'b}$ = 5.3 Hz); 1.84 (s, 3H, 5-CH3). Anal. calcd. for $C_{10}H_{15}N_3O_4S$•0.4 H_2O: C, 42.81; H, 5.68; N, 14.98. Found: C, 42.48; H, 5.40; N, 15.31.

7α ^1H NMR (Me_2SO-d_6) δ 7.75 (s, 1H, H-6); 7.29 (bs, 1H, NH); 6.80 (bs, 1H, NH); 5.87 (d, 1H, H-1', $J_{1',2'}$ = 7.7 Hz); 5.55 (d, 1H, 2'-OH, $J_{2',2'\text{-}OH}$ = 6.2Hz); 5.49 (d, 1H, 3'-OH, $J_{3',3'\text{-}OH}$ = 5.1 Hz); 4.88 (dd, 1H, 5'-OH, $J_{5'a,5'\text{-}OH}$ = 5.8 Hz, $J_{5'b,5'\text{-}OH}$ = 4.4 Hz); 3.97 (ddd, 1H, H-2', $J_{1',2'}$ = 7.7 Hz, $J_{2',3'}$ = 8.0 Hz, $J_{2',2'\text{-}OH}$ = 6.2 Hz); 3.84 (ddd, 1H, H-5'b, $J_{4',5'b}$ = 3.5 Hz, $J_{5'a,5'b}$ = 10.8 Hz, $J_{5'b,5'\text{-}OH}$ = 4.4 Hz); 3.60 (ddd, 1H, H-3', $J_{2',3'}$ = 8.0 Hz, $J_{3',3'\text{-}OH}$ = 5.1 Hz, $J_{3',4'}$ = 8.1 Hz); 3.57 (ddd, 1H, H-4', $J_{3',4'}$ = 8.1 Hz, $J_{4',5'b}$ = 3.5 Hz, $J_{4',5'a}$ = 8.2 Hz); 3.36 (ddd,

1H, H-5'a, $J_{4',5'a}$ = 8.2 Hz, $J_{5'a,5'b}$ = 10.8 Hz, $J_{5'a,5'-OH}$ = 5.8 Hz); 1.88 (s, 3H, 5-CH3). Anal. calcd. for $C_{10}H_{15}N_3O_4S$•0.4 H_2O: C, 42.81; H, 5.68; N, 14.98. Found: C, 42.52; H, 5.51; N, 15.38.

5-Chloro-1-(2,3,5-tri-*O*-benzyl-4-thio-α,β-D-arabinofuranosyl)uracil (8). This compound was prepared by the same procedure used for **2**, but on a 10 mmol scale substituting 5-chlorouracil (1.83 g, 12.5 mmol). The chromatographic purification afforded **8** (3.25 g, 58%) as a colorless syrup TLC (97:3 $CHCl_3$/MeOH) R_f 0.55; as 2:1 α,β mixture; MS z/e 570 $(M+Li)^+$.

^{1}H NMR ($CDCl_3$) δ 8.76 (s, 1H, H-6_β); 8.16 (bs, 2H, H-$3_{\alpha,\beta}$); 8.08 (s, 1H, H-6_α); 7.10 - 7.40 (m, 30H, aromatic H's); 6.30 (d, 1H, H-$1'_\beta$, $J_{1',2'}$ = 5.1 Hz); 6.14 (d, 1H, H-$1'_\alpha$, $J_{1',2'}$ = 1.8 Hz); 4.40-4.80 (overlapping multiplets, 12H, $C_6H_5CH_2$); 4.10-4.30 (m, 4H, H-$2_{\alpha,\beta}$ and H-$3_{\alpha,\beta}$); 3.96 (m, 1H, H-$4'_\alpha$); 3.50-3.76 (m, 4H, H-$5'_{\beta,\alpha}$); 3.40 (m, 1H, H-$4'_\beta$).

5-Chloro-1-(2,3,5-tri-*O*-benzyl-4-thio-β-D-arabinofuranosyl)cytosine (10β) and α anomer (10α). To a solution of **8** (360 mg, 0.64 mmol) in dry acetonitrile (10 mL) was added 4-DMAP (16.5 mg, 0.13 mmol) and 2,4,6-triisopropylbenzenesulfonyl chloride (995 mg, 3.19 mmol). This solution was cooled to 5 °C and triethylamine (0.45 mL, 3.24 mmol) was added dropwise over 5 min. The stirring was continued at room temperature for 7 h, at which time no starting material remained (TLC, cyclohexane/ethyl acetate 85:15). The reaction mixture was evaporated, dissolved in CH_2Cl_2 (10 mL), chilled to 5 °C, and mixed with ice-cold saturated aqueous NH_4Cl (15 mL). After being stirred for 15 min, the layers were separated. The aqueous layer was extracted twice with CH_2Cl_2 and the combined organic layers were washed twice with aqueous NH_4Cl, dried ($MgSO_4$), and evaporated to an orange foam **9** (R_f: **9α** 0.48 and **9β** 0.35, 95:5 cyclohexane:ethyl acetate); MS 837 (M+Li)+. The anomeric mixture was resolved on a flash column with 95:5 cyclohexane:ethyl acetate as solvent to provide **9α** (199 mg) and **9β** (127 mg) as white foams that were dissolved separately in 2.5:1 CH_3CN/conc. NH_4OH (28 μl/mg). After 16 h at room temperature, the reactions were evaporated. The resulting residues were purified by preparative TLC ($CHCl_3$:MeOH 95:5). The product bands were extracted with $CHCl_3$: MeOH 1:1 and extracts were evaporated to give the title

compounds: **10α** (white foam, 118 mg, 33%), TLC (95:5 $CHCl_3$:MeOH) R_f0.50, MS 570 (M+Li)+; and **10β** (white foam, 75 mg, 21%), TLC (95:5 $CHCl_3$:MeOH) R_f0.50, MS 570 (M+Li)+.

10β ^{1}H NMR ($CDCl_3$) δ 8.64 (s, 1H, H-6); 7.14-7.40 (m, 15H, aromatic H's); 6.58 (d, 1H, H-1′, $J_{1',2'}$ = 5.5 Hz); 6.30 (bs, 1H, NH); 5.54 (bs, 1H, NH); 4.40-4.72 (m, 6H, benzyl CH_2's); 4.14-4.28 (m, 2H, H2′ and H-3′); 5.44 (d, 1H, H-5); 3.56-3.72 (m, 2H, H-5′); 3.45 (m, 1H, H-4′).

10α ^{1}H NMR ($CDCl_3$) δ 8.20 (s, 1H, H-6); 7.08-7.40 (m, 15H, aromatic H's); 6.80 (bs, 1H, NH); 6.30 (d, 1H, H-1′, $J_{1',2'}$ = 0 Hz); 5.50 (bs, 1H, NH); 4.28-4.98 (m, 6H, benzyl CH_2's); 4.24 (bs, 1H, H-3′); 4.18 (bs, 1H, H-2′); 3.98 (m, 1H, H-4′); 3.78-3.82 (m, 1H, H-5′); 3.52-3.60 (m, 1H, H-5′).

5-Chloro-1-(4-thio-β-D-arabinofuranosyl)cytosine (11β) and α anomer (11α). The same procedure as for **5α/5β** was employed for **11β** starting from 289 mg (0.51 mmol) of **10β**. In this procedure, 8 mL (8 mmol) of the boron trichloride solution was used, and **10β** was dissolved in 8 mL of CH_2Cl_2. The cation exchange column provided the desired compound **11β** (84 mg, 56%); m.p. 215 °C (dec) (boiling EtOH); TLC (2:1:0.1 $CHCl_3$:MeOH:NH_4OH) R_f 0.42; HPLC 98%, 0.01M $NH_4H_2PO_4$:MeOH 4:1 (pH 5.1); MS z/e 294 $(M+H)^+$; UV λmax pH 1, 220 (12.3), 302 (11.7); pH 7, 220 (12.2), 291 (8.14); pH 13, 229 (8.64), 293 (8.67).

11β ^{1}H NMR (Me_2SO-d_6) δ 8.25 (s, 1H, H-6); 7.83 (bs, 1H, NH); 7.21 (bs, 1H, NH); 6.26 (d, 1H, H-1′, $J_{1',2'}$ = 4.8 Hz); 5.71 (d, 1H, 2′-OH, $J_{2',2'\text{-OH}}$ = 5.2Hz); 5.42 (d, 1H, 3′-OH, $J_{3',3'\text{-OH}}$ = 5.0 Hz); 5.25 (bt, 1H, 5′-OH, $J_{5'a,5'\text{-OH}}$ = $J_{5'b,5'\text{-OH}}$ = 4.9 Hz); 4.02-3.94 (m, 2H, H-2′, H-3′); 3.73 (dd, 1H, H-5′b, $J_{4',5'b}$ = 5.4 Hz, $J_{5'a,5'b}$ = 11.1 Hz, $J_{5'b,5'\text{-OH}}$ = 4.9 Hz); 3.64 (ddd, 1H, H-5′a, $J_{4',5'a}$ = 6.2 Hz, $J_{5'a,5'b}$ = 11.1 Hz, $J_{5'a,5'\text{-OH}}$ = 4.9 Hz); 3.18 (ddd, 1H, H-4′, $J_{3',4'}$ = 3.6 Hz, $J_{4',5'a}$ = 6.2 Hz, $J_{4',5'b}$ = 5.4 Hz). Anal. calcd. for $C_9H_{12}ClN_3O_4S$: C, 36.80; H, 4.12; N, 14.31. Found: C, 36.80; H, 3.96; N, 14.54.

Compound **11α** was prepared by the same method described for **11β** starting from **10α** (214 mg, 0.38 mmol). This compound (69 mg, 62%) was obtained as a white solid after crystallization from boiling water: **11α**, m.p. 235 °C (dec.); TLC (2:1:0.1

$CHCl_3$:MeOH:NH_4OH) R_f 0.35; HPLC 99%, 0.01M $NH_4H_2PO_4$:MeOH 4:1 (pH 5.1); MS z/e 294 $(M+H)^+$; UV λmax pH 1, 219 (12.6), 300 (11.2); pH 7, 220 (12.0), 290 (8.22); pH 13, 229 (8.27), 291 (8.16).

11α ^1H NMR (Me_2SO-d_6) δ 8.29 (s, 1H, H-6); 7.87 (bs, 1H, NH); 7.25 (bs, 1H, NH); 5.81 (d, 1H, H-1′, $J_{1',2'}$ = 6.6 Hz); 5.67 (d, 1H, 2′-OH, $J_{2',2'\text{-OH}}$ = 5.5Hz); 5.50 (d, 1H, 3′-OH, $J_{3',3'\text{-OH}}$ = 4.6 Hz); 4.93 (dd, 1H, 5′-OH, $J_{5'a,5'\text{-OH}}$ = 6.0 Hz, $J_{5'b,5'\text{-OH}}$ = 4.5 Hz); 4.05 (ddd, 1H, H-2′, $J_{1',2'}$ = 6.6 Hz, $J_{2',3'}$ = 6.9 Hz, $J_{2',2'\text{-OH}}$ = 5.5 Hz); 3.81 (ddd, 1H, H-5′b, $J_{4',5'b}$ = 4.4 Hz, $J_{5'a,5'b}$ = 10.8 Hz, $J_{5'b,5'\text{-OH}}$ = 4.5 Hz); 3.69 (dd, 1H, H-3′, $J_{2',3'}$ = 6.9 Hz, $J_{3',3'\text{-OH}}$ = 4.6 Hz, $J_{3',4'}$ = 8.2 Hz); 3.56 (ddd, 1H, H-4′, $J_{3',4'}$ = 8.2 Hz, $J_{4',5'b}$ = 4.4 Hz, $J_{4',5'a}$ = 6.9 Hz); 3.40 (ddd, 1H, H-5′a, $J_{4',5'a}$ = 6.9 Hz, $J_{5'a,5'b}$ = 10.8 Hz, $J_{5'a,5'\text{-OH}}$ = 6.0 Hz). Anal. calcd. for $C_9H_{12}ClN_3O_4S$: C, 36.80; H, 4.12; N, 14.31. Found: C, 36.52; H, 3.97; N, 14.28.

Acknowledgment. This investigation was supported by NIH Grant No. CA34200. We gratefully acknowledge the data received from the following laboratories at Southern Research Institute: Ms. Sheila Campbell (HPLC analyses), Mr. Jerry Frye (large-scale synthesis), and Dr. James M. Riordan, Mr. Mark Richardson, and Ms. Joan Bearden of the Spectroscopic and Analytical Laboratory for spectral and analytical data. The assistance of Ms. Stephanie Brannon in the preparation of this manuscript is appreciated.

REFERENCES

1. Montgomery, J.A. Antimetabolites. In *Cancer Chemotherapeutic Agents*; Foye, W.O., Ed.; American Chemical Society: Washington, D.C., 1995, 47-109.
2. Secrist, J.A., III; Tiwari, K.N.; Riordan, J.M.; Montgomery, J.A. *J. Med. Chem.*, **1991**, *34*, 2361-2366.
3. Tiwari, K.N.; Montgomery, J.A.; Secrist, J.A., III. *Nucleosides Nucleotides,* **1993**, *12*, 841-846.
4. Secrist, J.A., III; Riggs, R.M.; Tiwari, K.N.; Montgomery, J.A. *J. Med. Chem.*, **1992**, *35,* 533-538.
5. Tiwari, K.N.; Secrist, J.A., III; Montgomery, J.A. *Nucleosides Nucleotides*, **1994**, *13*, 1819-1828.

6. Secrist, J.A., III; Parker, W.B.; Tiwari, K.N.; Messini, L.; Shaddix, S.C.; Rose, L.M.; Bennett, L.L., Jr.; Montgomery, J.A. *Nucleosides Nucleotides*, **1995**, *14*, 675-686.
7. Secrist, J.A., III; Tiwari, K.N.; Shortnacy-Fowler, A.T.; Messini, L.; Riordan, J.M.; Montgomery, J.A. *J. Med. Chem.*, **1998**, *41*, 3865-3871.
8. Tiwari, K.N.; Shortnacy-Fowler, A.T.; Cappellacci, L.; Parker, W.B.; Waud, W.R.; Montgomery, J.A.; Secrist, J.A., III. *Nucleosides, Nucleotides & Nucleic Acids*, **2000**, *19*, 329-340.
9. Schiller, J. H.; Groveman, D. S.; Schmid, S. M.; Wilson, J.K.V.; Cummings, K.B.; Borden, E.C. *Cancer Res.*, **1986**, *46*, 483-488.
10. Struck, R. F.; Schmid, S.M.; Waud, W.R. *Cancer Chemother. Pharmacol.*, **1994**, *34*, 191-196.
11. Waud, W.R.; Tiwari, A.; Schmid, S.M.; Shih, T.-W.; Strong, J.M.; Hartman, N.R.; O'Reilly, S.; Struck, R.F. *Cancer Res.*, **1997**, *57*, 815-817.
12. Waud, W.R.; Gilbert, K.S. Shepherd, R.V.; Harwell, J.E.; Tiwari, K.N.; Secrist, J.A., III; Montgomery, J.A. Ab. No. 1972, Vol. 40, 1999. Proceedings of 90th Annual Meeting of the Am. Assoc. for Cancer Res., Philadelphia, PA April 10-14, 1999.
13. Awano, H.; Shuto, S.; Baba, M.; Kira, T.; Shigeta, S.; Matsuda, A. *Bioorg. Med. Chem. Lett.*, **1994**, *4*, 367-370.