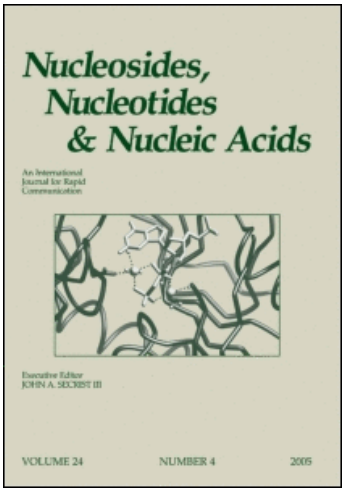

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Synthesis and Anti-cancer Activity of Some Novel 5-Azacytosine Nucleosides[#]

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ABSTRACT

1-*O*-Acetyl-2-deoxy-3,5-di-*O*-toluoyl-4-thio-D-*erythro*-pentofuranose and 2-deoxy-1,3,5-tri-*O*-acetyl-4-thio-L-*threo*-pentofuranose were coupled with 5-azacytosine to obtain α and β anomers of nucleosides. All four nucleosides were reduced to the corresponding dihydro derivatives and deblocked to give target compounds. All eight target compounds were evaluated in a series of human cancer cell lines in culture. Only 2′-deoxy-4′-thio-5-azacytidine (**3β**) was found to be cytotoxic in all the cell lines and was further evaluated in vivo. Details of the synthesis and biological activity are reported.

Key Words: 5-Azacytosine; 4′-Thionucleoside; Anti-cancer activity.

[#]In honor and celebration of the 70th birthday of Professor Leroy B. Townsend.

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INTRODUCTION

A number of 2′-deoxyribo and arabino nucleoside analogs have shown promise as anticancer agents. For some years we have been pursuing the synthesis of a variety of 4′-thionucleosides as anticancer agents. In that regard, we have prepared 4′-thionucleosides with a variety of different carbohydrates attached to both the normal purine and pyrimidine bases as well as analogs of those bases.[1–7,14] We have reported the synthesis and antitumor activity of 1-(4-thio-β-D-arabinofuranosyl) cytosine (4′-thio-ara-C), which is currently in clinical trials. Preliminary results on the mechanism of action of 4′-thio-ara-C suggest that it is metabolized to its triphosphate using deoxycytidine kinase as the first step, and that the triphosphate inhibits DNA polymerase and is also incorporated into DNA.[8] The activity of 4′-thio-ara-C has encouraged us to continue research on related compounds.

One of the altered bases that has drawn attention for many years is 5-azacyosine. This base, when present in 5-azacytidine,[11] 2′-deoxy-5-azacytidine,[10] and ara-5-azacytidine,[13] imparts anticancer activity, and these compounds have been examined clinically.[9] 5-Azacytidine, for example, after conversion to the corresponding triphosphate by cellular kinases, is incorporated into RNA. It is also incorporated into DNA after reduction at the diphosphate level to the 2′-deoxynucleoside. In cells in culture, 5-azacytidine has little effect on resting cells, being more toxic to cells in the S phase of the cell cycle, indicating that its primary effect relates to DNA synthesis.[10] Incorporation of 2′-deoxy-5-azacytidine into DNA results in hypomethylation of the DNA, as well as other methylation-related effects. Though insufficient anticancer selectivity was found with these compounds, it is clear that some of the biological effects caused by the 5-azacytosine moiety are quite desirable, if they can be incorporated into a molecule with other favorable properties.

A problem associated with certain 5-azacytidine analogs, including those mentioned above, is the ready hydrolysis by ring opening of the base moiety in aqueous solution. In order to combat that problem, Beisler et al. prepared the 5,6-dihydro analog of 5-azacytidine,[11] which is much more stable hydrolytically, and examined it for anticancer activity. It was found to have activity, though only at about one-tenth of 5-azacytidine. Mechanistically, its activation and mode of action appeared to be similar to those of 5-azacytidine.[12]

On the basis of the above information, we have prepared a series of 4′-thionucleosides incorporating both 5-azacytosine and 5,6-dihydro-5-azacytosine, in order to assess their anticancer activity, as well as to compare the saturated and unsaturated compounds in the same system. Specifically, we have prepared both the β and α anomers of the nucleosides incorporating 5-azacytosine and 5,6-dihydro-5-azacytosine into 4-thio-2-deoxy-L-*threo*-pentofuranose and 4-thio-2-deoxy-D-*erythro*-pentofuranose. Chemical synthesis and biological data on these compounds are presented herein.

CHEMISTRY

The synthesis of 1-*O*-acetyl-2-deoxy-4-thio-3,5-di-*O*-p-toluoyl-D-*erythro*-pentofuranose **1** and 2-deoxy-1,3,5-*O*-acetyl-4-thio-L-*threo*-pentofuranose **6**, as 1:1 mixture of anomers have been performed as previously reported.[3,14]

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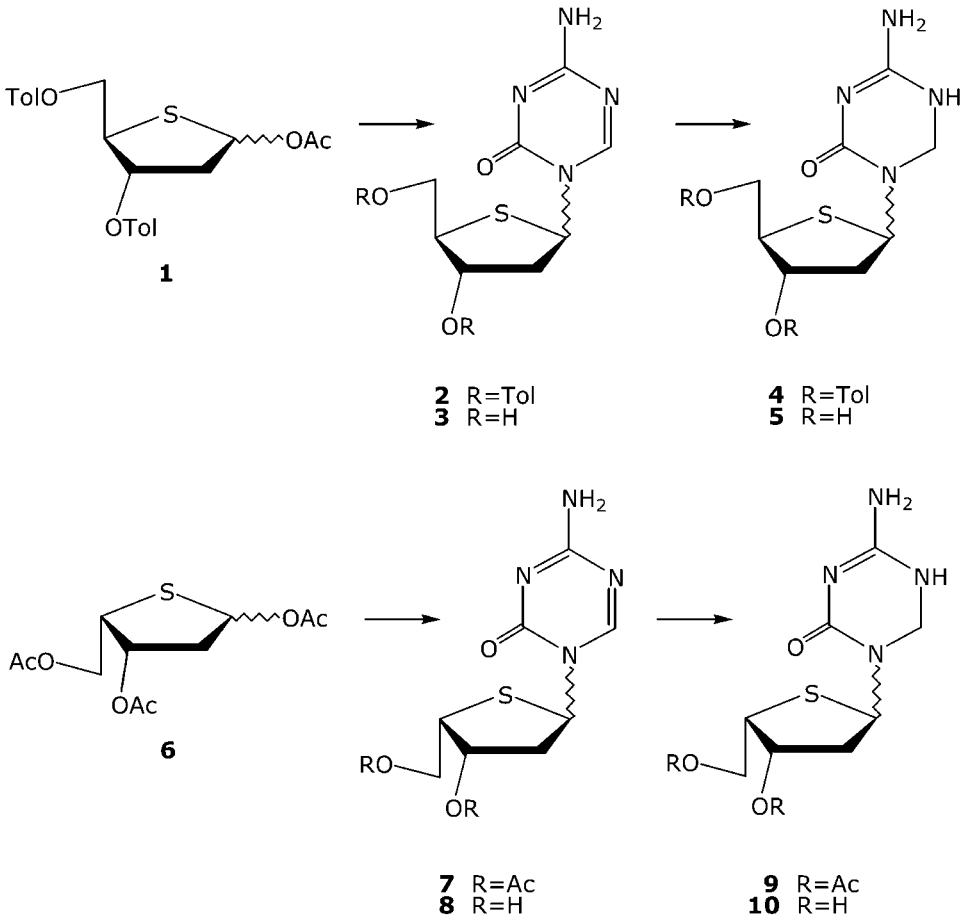

Scheme 1.

Trimethylsilyl triflate catalyzed coupling[3,14] of thiosugar **1** and **6** with silylated 5-azacytosine afforded the corresponding nucleosides (**2** and **7**) as anomeric mixtures (β:α ratio ~1:2 and 2:1 respectively). Silica gel chromatography and fractional crystallization of **2** and **7** afforded pure anomers. Reduction of the 5,6-double bond with sodium borohydride of compounds **2α**, **2β**, **7α**, **7β**, afforded **4α**, **4β**, **9α**, **9β**, respectively. Deprotection of compounds **2α**, **2β**, **7α**, **7β**, **4α**, **4β**, **9α**, **9β**, with sodium methoxide afforded **3α**, **3β**, **8α**, **8β**, **5α**, **5β**, **10α**, **10β**, respectively (Sch. 1). We found, as previously seen with other 5-azacytosine-containing nucleosides,[11] that **3α**, **3β**, **8α**, and **8β** all were somewhat unstable in solution. After reduction of the 5,6 double bond, however, stability was restored.

The anomeric configuration and point of attachment of sugar and pyrimidine in all the nucleosides were confirmed by ^{1}H NMR spectra using decoupling experiments.[3,14]

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Table 1. Cytotoxicity data: IC50 (μM).

Compound	CCRF-CEM (leukemia)	CAKI-1 (renal)	DLD-1 (colon)	NCI-H23 (lung)	LOXIMVI (melanoma)	SNB-7 (CNS)
3β	0.01	13.0	7.0	3.1	5.3	1.7

BIOLOGICAL DATA

The cytotoxicity of all eight analogs was determined against a panel of human tumor cell lines.[15] Only **3β** was cytotoxic to these cell lines (Table 1). The other agents were not cytotoxic at the highest level tested (60 μM). On the basis of these results, larger quantities of **3β** were prepared for evaluation in an animal model. The animal model chosen was the NCI-H23 non-small cell lung tumor implanted subcutaneously in female nude mice. H23 was chosen because it is a solid tumor model, rather than a leukemia, and because it is sensitive to a variety of agents.[16,17] This model also has previously been shown in our laboratories to be sensitive to 4′-thio-ara-C.[18] In this study treatment began on day 15 when the median tumor size ranged from 162 to 188 mg. Compound **3β** was prepared in saline/tween 80 and was administered intraperitoneally as a daily single injection on days 15–23. Modest activity was observed at a tolerated dosage of 4.5 mg/kg/dose. Treated animals had an increase in lifespan of 8 days beyond the control animals (T-C value of 8 days). Because of the inherent instability of this compound in solution, the same study was performed in which compound **3β** was prepared fresh daily. In this study, similar modest activity was observed at a tolerated dosage of 6.7 mg/kg/dose (T-C value of 10.9 days).

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). Chemical shifts are expressed in parts per million downfield from tetramethylsilane. The NOE experiments were conducted on a degassed solution of DMSO-d_6. To minimize the effects of magnetic perturbations with the sample non-spinning, eight FIDs were acquired with the decoupler set to a desired frequency and eight FIDs were recorded with the decoupler off resonance. The process was repeated until 800 FIDs had been acquired. UV absorption spectra were determined with a Perkin-Elmer λ 9 spectrometer by dissolving each compound in methanol or water and diluting 10-fold with 0.1 N HCl, pH 7 buffer, or 0.1 N NaOH. Numbers in parentheses are extinction coefficients ($\times 10^{-3}$), sh = shoulder. Microanalyses were performed by Atlantic Microlab, Inc. (Atlanta, GA) or 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

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(4.6 mm × 25 cm) 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 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}.

1-(2-Deoxy-3,5-di-*O*-toluoyl-4-thio-β-D-*erythro*-pentofuranosyl)-5-azacytosine (2β) and Its α-Anomer (2α). To a suspension of 1-*O*-acetyl-3,5-di-*O*-toluoyl-4-thio-D-erythropentofuranose (428 mg, 1 mmol) and 5-azaytosine (140.0 mg, 1.25 mmol) in anhydrous acetonitrile (20 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, after which time the reaction was essentially complete. The mixture was warmed to room temperature, concentrated to a small volume (5 mL), diluted with methylene chloride (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 chromatography over silica gel (50 g. elution with $CHCl_3$/MeOH 98:2) to afford **2** (336 mg, 70%) as a colorless syrup TLC (95:5 $CHCl_3$/MeOH R_f 0.45; as 2:1 α,β mixture (HPLC); MS m/z 481 $(M+H)^+$. Both the anomers were separated by silica gel column using 200:1 ratio of silica gel and compound to obtain **2β** (100 mg) and **2α** (200 mg) as white solids. **2β** MS m/z 481 $(M+H)^+$. ^1H NMR ($CDCl_3$) 8.60 (s, 1H, H-6), 7.96 (d, 2H, ortho H of toluoyl, J = 8 Hz), 7.94 (d, 2H, ortho H of toluoyl, J = 8.0 Hz), 7.62 (s, 2H, NH_2), 7.34 (t, 4H, meta H of toluoyl, J = 4 Hz), 6.20 (dd, 1H, H-1′, J = 8 and 1 Hz), 5.78–5.80 (m, 1H, H-3′), 4.60–4.64 (m, 1H, H-5′), 4.46–4.50 (m, 1H, H-5′), 3.90–3.96 (m, 1H, H-4′), 2.92–3.0 (m, 1H, H-2′), 2.60–2.68 (m, 1H, H-2′), 2.40 (s, 3H, CH_3), 2.38 (s, 3H, CH_3).

2α MS m/z 481 $(M+H)^+$. ^1H NMR ($CDCl_3$) 8.80 (s, 1H, H-6), 7.92 (d, 2H, ortho H of toluoyl, J = 8 Hz), 7.76 (d, 2H, ortho H of toluoyl, J = 8.0 Hz), 7.54 (d, 2H, NH_2, J = 4 Hz), 7.36 (d, 2H, meta H of toluoyl, J = 8 Hz), 7.30 (d, 2H, meta H of toluoyl, J = 8 Hz), 6.10 (dd, 1H, H-1′, J = 4 and 2 Hz), 5.66–5.70 (m, 1H, H-3′), 4.26–4.44 (m, 3H, H-4′, H-5′), 2.82–2.86 (m, 1H, H-2′), 2.64–2.68 (m, 1H, H-2′), 2.20 (s, 3H, CH_3), 2.18 (s, 3H, CH_3).

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1-(2-Deoxy-3,5-di-*O*-acetyl-4-thio-β-L-*threo*-pentofuranosyl)-5-azacytosine (7β) and Its α-Anomer (7α). To a suspension of 1-*O*-acetyl-3,5-di-*O*-acetyl-4-thio-L-thropentofuranose (276 mg, 1 mmol) and 5-azaytosine (140.0 mg, 1.25 mmol) in anhydrous acetonitrile (20 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 2.0 h, after which time the reaction was essentially complete. The mixture was warmed to room temperature, concentrated to a small volume (5 mL), diluted with methylene chloride (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 chromatography over silica gel (50 g. elution with $CHCl_3$/MeOH 98:2) to afford **2** (262 mg, 80%) as a colorless syrup TLC (96:4 $CHCl_3$/MeOH R_f 0.50; as 2:1 α:β mixture (HPLC); MS m/z 329 $(M+H)^+$. Both the anomers were separated by silica gel column using using 200:1 ratio of silica gel and compound to obtain **7β** (160 mg) and **7α** (75 mg) as white solids. **7β** MS m/z 329 $(M+H)^+$. ^1H NMR ($CDCl_3$) 8.90 (s, 1H, H-6), 6.21 (t, 1H, H-1′, J = 3.0 Hz), 5.64–5.70 (overlapping multiplets, 2H, H-3′, H of NH_2), 5.42 (broad hump, 1H, H of NH_2), 4.44–4.50 (m, 1H, H-5′), 4.26–4.32 (m, 1H, H-5′), 4.0–4.06 (m, 1H, H-4′), 2.54–2.56 (m, 2H, H-2′), 2.08 (s, 3H, CH_3), 1.98 (s, 3H, CH_3).

7α MS m/z 329 $(M+H)^+$. ^1H NMR ($CDCl_3$) 8.52 (s, 1H, H-6), 6.70 (bm, 1H, H-1′), 6.32 (t, 1H, H of NH_2, J = 6 Hz), 5.70 (bs, 1H, H-6), 5.54–5.60 (m, 1H, H of NH_2), 4.31–4.36 (m, 1H, H-5′), 4.14–4.26 (m, 2H, H-4′, H-5′), 2.76–2.84 (m, 1H, H-2′), 2.24–2.34 (m, 1H, H-2′), 2.12 (s, 3H, CH_3), 2.08 (s, 2H, CH_3).

1-(2-Deoxy-4-thio-β-D-*erythro*-pentofuranosyl)-5-azacytosine (3β) and Its α-Anomer (3α). To a solution of **2β** (60 mg, 0.125 mmol) in anhydrous MeOH (30 mL) was stirred at room temperature with a freshly prepared solution of sodium methoxide (20 mg, 0.37 mmol) in MeOH (20 mL). A TLC aliquot (3 h, $CHCl_3$: MeOH, 85:15) showed complete consumption of starting material. The solution was rendered neutral with Dowex 50W*8 (H+) ion exchange resin, and the resin was filtered off with MeOH washing. The filtrate were combined and evaporated to dryness. Preparative TLC ($CHCl_3$:MeOH, 9:1) of the crude mixture gave white solid, which was crystallized with ethanol to obtain **3β** (15 mg, 50%), m.p. 211°C; MS m/z 245 (M + H), ^1H NMR (DMSO-*d*6) 8.70 (s, 1H, H-6); 7.54 (s, 2H, NH_2), 6.08 (t, 1H, H-1′, J = 6.0), 5.31 (d, 1H, H-3′, J = 4 Hz), 5.16 (t, 1H, 5′-OH, J = 4 Hz), 4.30–4.34 (m, 1H, H-3′), 3.50–3.66 (m, 2H, 5′-H), 3.22–3.34 (m, 1H, H-4′), 2.16–2.32 (m, 2H, H-2′). Anal. calcd for $C_8H_{12}N_4O_3S$: C, 39.33; H, 4.95; N, 22.95. Found: C, 39.26; H, 4.84; N, 22.68.

Compound **3α** was also made using the same procedure in 80% yield starting from 150 mg of **2α** m.p. 223°C; MS m/z 245 $(M+H)^+$, ^1H NMR (DMSO-*d*6) 8.80 (s, 1H, H-6), 7.42 (d, 2H, NH_2, J = 1.5 Hz), 6.0 (dd, 1H, H-1′, J = 4.0 and 2.0 Hz), 5.40 (d, 1H, 3′-OH, J = 2 Hz), 5.05 (t, 1H, 5′-OH, J = 2 Hz), 4.40–4.44 (m, 1H, H-3′), 3.54–3.60 (m, 1H, H-4′), 3.30–3.42 (m, 2H, 5′-H), 2.34–2.44 (m, 1H, H-2′), 2.14–2.20 (m, 1H, H-2′). Anal. calcd for $C_8H_{12}N_4O_3S$: 0.1 H_2O C, 39.04; H, 5.0; N, 22.78. Found: C, 38.99; H, 4.84; N, 22.62.

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1-(2-Deoxy-4-thio-β-L-*threo*-pentofuranosyl)-5-azacytosine (8β) and Its α-Anomer (8α). To a solution of **7β** (60 mg, 0.183 mmol) in anhydrous MeOH (30 mL) was stirred at room temperature with a freshly prepared solution of sodium methoxide (20 mg, 0.37 mmol) in MeOH (20 mL). A TLC aliquot (3 h, $CHCl_3$:MeOH, 85:15) showed complete consumption of starting material. The solution was rendered neutral with Dowex 50W*8 (H+) ion exchange resin, and the resin was filtered off with MeOH washington. The filtrate were combined and evaporated to dryness. Preparative TLC ($CHCl_3$:MeOH, 9:1) of the crude mixture gave white solid, which was crystallized with ethanol to obtain **8β** (33 mg, 74%), m.p. 211°C; MS m/z 245$(M+H)^+$. ^{1}H NMR (DMSO-*d*6) 8.84 (s, 1H, H-6), 7.44 (d, 2H, NH_2, J = 6 Hz), 6.60 (dd, 1H, H-1′, J = 8.0 and 2.0 Hz), 5.31 (d, 1H, H-3′, J = 4 Hz), 4.90–4.94 (m, 1H, 5′-OH), 4.34–4.42 (m, 1H, H-3′), 3.84–3.90 (m, 1H, 5′-H), 3.60–3.68 (m, 2H, H-4′, H-5′), 2.20 2.34 (m, 2H, H-2′). Anal. calcd for $C_8H_{12}N_4O_3S$: 0.1 H_2O, 0.1 C_2H_5OH C, 39.28; H, 5.15; N, 22.36. Found: C, 39.32; H, 4.82; N, 22.35.

Compound **8α** was also made using the same procedure in 82% yield starting from 150 mg of **7α** m.p. 223°C; MS m/z 245 $(M+H)^+$, ^{1}H NMR (DMSO-*d*6) 8.64 (s, 1H, H-6), 7.44 (s, 2H, NH_2), 6.20 (dd, 1H, H-1′, J = 12.0 and 8.0 Hz), 5.10 (d, 1H, H-3′, J = 4 Hz), 4.78–4.84 (m, 1H, 5′-OH), 4.40–4.44 (m, 1H, H-3′), 4.90–4.98 (m, 1H, H-4′), 3.70–3.80 (m, 1H, 5′-H), 3.40–3.48 (m, 1H, H-5′), 2.22–2.34 (m, 2H, H-2′). Anal. calcd for $C_8H_{12}N_4O_3S$: 0.1 H_2O C, 39.04; H, 5.0; N, 22.78. Found C, 38.27; H, 4.84; N, 22.41.

1-(2-Deoxy-4-thio-β-D-*erythro*-pentofuranosyl)-5,6-dihydro-5-azacytosine (5β) and Its α-Anomer (5α). A solution of **2β** (96 mg, 0.20 mmol) in dry THF (20 mL) was added solid sodium borohydride (64 mg, 1.68 mmol), and the mixture was stirred at r.t. One hour aliquot showed complete reaction (CH3CN:NH4OH, 9:1). The solvent was evaporated and the residue was diluted with cold MeOH (5 mL). The solution was quickly treated was Dowex 50W*8 strong cation exchange resin until neutrilization. The resin was filtered, washed with MeOH and the filtrate was evaporated to dryness to give crude which was purified by a small silica gel column to afford pure **4β** (80 mg, 80%) as white powder MS m/z 483 $(M+H)^+$. This compound was deblocked in a similar manner as described for compound **3** to afford compound **5β** (white solid, 78%) and **5α** (white solid, 70%) was made in a similar manner from **3α**. Compound **5β** MS m/z 247 $(M+H)^+$. ^{1}H NMR (DMSO-*d*6) 6.24 (dd, 1H, H-1′, J = 8 and 1 Hz), 5.10 (bs, 1H, 3′-OH), 5.12 (bs, 1H, 5′-OH), 4.60 (d, 1H, H-6, J = 6 Hz), 4.42 (d, 1H, H-6, J = 6 Hz), 4.28 (s, 1H, H-3′), 3.40–3.42 (m, 1H, H-5′), 3.10–3.14 (m, 1H, H-4′), 2.0–1.98 (m, 1H, H-2′), 1.60–1.64 (m, 1H, H-2′). Anal. calcd for $C_8H_{14}N_4O_3S$: 0.1 H_2O, 0.1C_2H_5OH C, 38.97; H, 5.90; N, 22.18. Found: C, 38.96; H, 5.81; N, 22.29.

5α MS m/z 247 $(M+H)^+$. ^{1}H NMR (DMSO-*d*6) 6.08 (t, 1H, H-1′, J = 4 Hz), 5.24 (d, 1H, 3′-OH, J = 4 Hz), 4.82 (t, 1H, 5′-OH, J = 4 Hz), 4.62 (d, 1H, H-6, J = 6 Hz), 4.54 (d, 1H, H-6, J = 6 Hz), 3.90–3.96 (m, 1H, H-3′), 3.62–3.64 (m, 1H, H-4′), 3.24–3.44 (m, 1H, H-5′), 2.20–2.28 (m, 1H, H-2′), 1.80–1.90 (m, 1H, H-2′). Anal. calcd for $C_8H_{14}N_4O_3S$: 0.1 H_2O, 0.1C_2H_5OH C, 38.97; H, 5.90; N, 22.18. Found: C, 38.90; H, 5.60; N, 22.25.

1-(2-Deoxy-4-thio-β-L-*threo*-pentofuranosyl)-5,6-dihydro-5-azacytosine (10β) and Its α-Anomer (10α). Compound **10β** (white solid, 78%) and **10α** (white solid,

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80%) were made in a similar manner from **7β** and **7α** respectively as reported for compound **5**. **10β** MS m/z 247 $(M+H)^+$. ^{1}H NMR (DMSO-*d*6) 6.10 (dd, 1H, H-1′, J = 12.0 and 8.0 Hz), 4.70–4.90 (m, 2H, H-6), 4.44–4.50 (m, 1H, H-3′), 3.98–4.02 (m, 1H, 5′-H), 3.70–3.78 (m, 1H, H-5′), 3.54–3.58 (m, 1H, H-4′), 2.30–2.42 (m, 1H, H-2′), 2.04–2.14 (m, 1H, H-2′). Anal. calcd for $C_8H_{14}N_4O_3S$: 0.1 H_2O, 0.1C_2H_5OH C, 38.97; H, 5.90; N, 22.18. Found: C, 38.90; H, 5.98; N, 22.15.

10α MS m/z 247 $(M+H)^+$. ^{1}H NMR (DMSO-*d*6) 6.26 (dd, 1H, H-1′, J = 12.0 and 6.0 Hz), 4.90–5.00 (m, 1H, H-6), 4.70–4.85 (m, 1H, H-6), 4.55–4.65 (m, 1H, H-3′), 3.90–3.98 (m, 2H, H-4′, 5′-H), 3.60–3.70 (m, 1H, H-5′), 2.32–2.44 (m, 1H, H-2′), 2.06–2.16 (m, 1H, H-2′). Anal. calcd for $C_8H_{14}N_4O_3S$: 0.1 H_2O, 0.1C_2H_5OH C, 38.97; H, 5.90; N, 22.18. Found: C, 39.0; H, 5.75; N, 22.30.

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