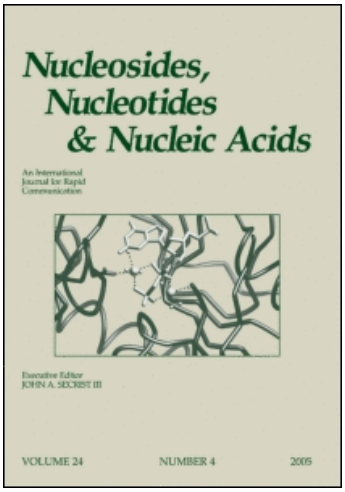

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 5-FLUORO-2-THIOCYTOSINE NUCLEOSIDES

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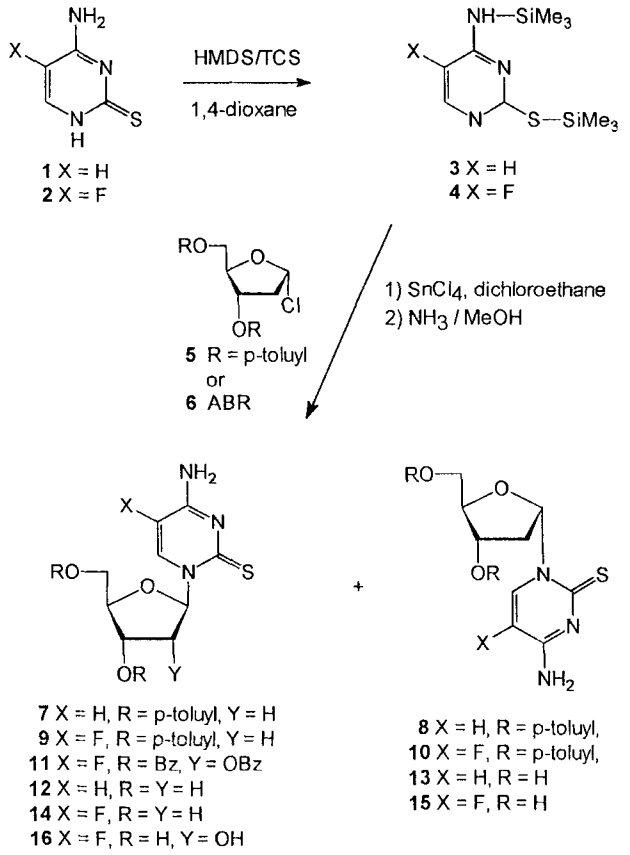
Abstract. Two pathways are described for the synthesis of the 2'-deoxynucleosides of 2-thiocytosine and 5-fluoro-2-thiocytosine: (a) by nucleoside condensation, (b) by amination of the corresponding nucleosides of 2,4-dithiouracil. Biological activities *vs* two cell systems are described. The nucleosides are moderate to weak substrates of deoxycytidine kinase and, partly as a result of this, reasonable good inhibitors of the enzyme

Our previous observation that the 5-fluoro-2'-deoxyuridine analogue, 5-fluoro-2-thio-2'-deoxyuridine, exhibits antileukemic activity, while its 5'-phosphate is a potent, slow-binding inhibitor of thymidylate synthase,[1] prompted us to undertake the synthesis of further analogues of this series, *viz*. 2-thio-2'-deoxycytidine, its 5-fluoro congener, as well as 5-fluoro-2-thiocytidine.

Various procedures were examined to obtain the appropriate base for the condensation reaction. Our previous experience on the use of Lawesson reagent (O.L.) for thiation of uracil analogues[2] suggested its possibly utility for thiation of cytosine and 5-fluorocytosine. Each of these, heated under reflux with an equimolar amount of the reagent in 1,4-dioxane for 16 h, gave the expected product in 90% yield. In the case of 5-fluoro-2-thiocytosine, the product crystallized on cooling. With cytosine the expected product proved difficult to isolate, and the recourse was then to selectively aminate 2,4-dithiouracil, prepared with the aid of the Lawesson reagent.[2]

Preparation of the nucleoside of 2-thiocytosine (**1**) by the condensation reaction required effective silylation of the latter. Application of standard conditions to 5-fluoro-2-thiocytosine (**2**), i.e. use of a 10:1 mixture of HMDS/TCS, hence a 10-fold excess of HMDS, and extended



SCHEME 1

heating under reflux, gave the silylated product **4** in a good yield, but was unsuccessful with 2-thiocytosine. Eventually the use of a 10-fold excess of a 1:1 mixture of HMDS/TCS led to a good yield of the silylated product (**3**).

The silylated bases **3** and **4** (Scheme 1) were each condensed with 1-chloro-3,5-di-O-p-toluyl-2-deoxyribofuranose (**5**) in anhydrous dichloroethane, with an equimolar amount of $SnCl_4$ as catalyst. This led to mixtures of ß- and α-anomers of 2',5'-di-O-p-toluyl-2'-deoxy-2-thiocytidine (**7** and **8**), and 3',5'-di-O-p-toluyl-2'-deoxy-5-fluoro-2-thiocytidine (**9** and **10**), with ß/α ~ 3:1. The ß-anomers **7** and **9** were isolated by fractional crystallization from ethanol, and the α-anomers **8** and **10** by preparative chromatography on silica gel plates with chloroform-acetone-ethyl acetate (85:10:5, v/v). The isolated anomers were deblocked with ammoniacal methanol to give the ß- and α-2'-deoxynucleosides of 2-thiocytosine (**12** and **13**) and ß- and α-2'-deoxynucleosides 5-fluoro-2-thiocytosine (**14** and **15**).

TABLE 1

Inhibition of cell growth by 2-thiocytosine nucleoside analogues [IC_{50} (M)]

Compound	L5178Y cells			3T3 cells		
	Growth inhibition	Colony formation	[^{14}C]Leu incorporation	Growth inhibition	Colony formation	[^{14}C]Leu incorporation
AraC	7×10^{-9}	8×10^{-9}	7×10^{-9}	7×10^{-8}	2×10^{-8}	5×10^{-9}
5FdCyd	5×10^{-6}	3×10^{-6}	8×10^{-7}	3.5×10^{-6}	3.5×10^{-6}	3×10^{-7}
2S5FCyd (**16**)	9×10^{-7}	5×10^{-7}	7×10^{-7}	5×10^{-5}	7×10^{-6}	8×10^{-6}
2SdCyd (**12**)	1.4×10^{-4}			1.8×10^{-4}		
α-2S5FdCyd (**13**)	3×10^{-5}	8×10^{-5}	5×10^{-5}	$> 10^{-3}$	5×10^{-4}	6×10^{-4}
ß-2S5FdCyd (**14**)	8×10^{-7}	2×10^{-6}	1×10^{-6}	1×10^{-4}	5.5×10^{-5}	5×10^{-6}

The nucleosides **12** and **14** were also obtained by thiation with the Lawesson reagent in dioxane of the corresponding previously synthesized blocked 3',5'-di-O-p-toluyl-2'-deoxy-2-thiouridines,[1] to give the corresponding blocked 2,4-dithionucleosides in a good yield. Amination with NH_3-MeOH at elevated temperature led to compounds **12** and **14**, identical to the same products obtained by condensation reactions, the structures of which were established by ^{1}H NMR spectroscopy (500 MHz).

For comparison of biological properties of the foregoing, 5-fluoro-2-thiocytidine (**16**) was synthesized as previously reported.[3]

BIOLOGICAL RESULTS

The effects of the foregoing new compounds on the growth, colony formation and protein synthesis ([^{14}C]leucine incorporation) of mouse leukemic L5178Y cells and mouse 3T3 fibroblast were examined as previously described[1] and compared with several known nucleoside analogues (see Table 1). IC_{50} values are expressed as the molar concentrations leading to a 50% reduction in cell count, colony formation and [^{14}C]leucine incorporation. The ß-anomer of **14** and ribonucleoside **16** were, in fact, more active than 5FdCyd *vs* mouse leukemic L5178Y cells; the α-anomer **13** was less active.

An examination was made of the substrate/inhibitor properties of **12** and **14** *vs* human leukemic spleen deoxycytidine kinase. Their enzymatic conversion to the corresponding 5'-phosphates was demonstrated by their identity with the 5'-phosphates of both nucleosides

TABLE 2

Transfer of γ-^{32}P from ATP to 2-thio-2'-deoxycytidine and related nucleosides by human leukemic spleen deoxycytidine kinase

Compound	Concentration (μM)	Transfer of γ-^{32}P from ATP (%)
dCyd	100	100
2SdCyd (**12**)	10	5
	100	20
ß-2S5FdCyd (**14**)	10	10
	100	10

prepared on a larger scale by phosphorylation with the wheat shoot nucleoside phosphotransferase system.[4] Nucleoside **12** was found to be a reasonable, and **14** even weaker substrate of human deoxycytidine kinase (Table 2).

Both **12** and **14** were also potent inhibitors of the phosphorylation of deoxycytidine and deoxyadenosine. The IC_{50} values for **12** were 1.0 μM and 0.08 μM, respectively; while **14** was a more potent inhibitor with IC_{50} values of 0.1 μM and 0.025 μM, respectively.

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