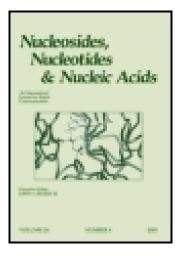
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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 5-FLUORO-2-THIOCYTOSINE NUCLEOSIDES

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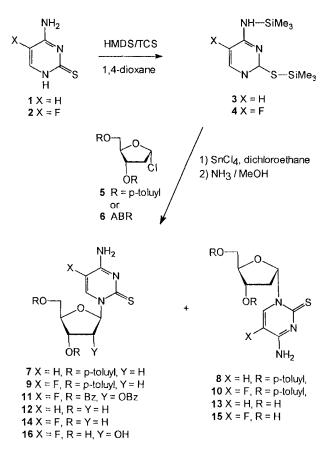
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Abstract. Two pathways are described for the synthesis of the 2'-deoxynucleosides of 2-thiocytosine and 5-fluoro-2-thiocytosine: (a) by nucleoside condensation, (b) by amination of the corresponding nucleosides of 2,4-dithiouracil. Biological activities 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-2thiocytidine.

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 analogues² 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 silulation 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





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

The silylated bases 3 and 4 (Scheme 1) were each condensed with 1-chloro-3,5-di-O-ptoluyl-2-deoxyribofuranose (5) in anhydrous dichloroethane, with an equimolar amount of SnCl₄ as catalyst. This led to mixtures of β - and α -anomers of 2',5'-di-O-p-toluyl-2'-deoxy-2thiocytidine (7 and 8), and 3',5'-di-O-p-toluyl-2'-deoxy-5-fluoro-2-thiocytidine (9 and 10), with $\beta/\alpha \sim 3:1$. The β -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 chloroformacetone-ethyl acetate (85:10:5, v/v). The isolated anomers were deblocked with ammoniacal methanol to give the β - and α -2'-deoxynucleosides of 2-thiocytosine (12 and 13) and β - and α -2'-deoxynucleosides 5-fluoro-2-thiocytosine (14 and 15).

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

TABLE 1Inhibition of cell growth by 2-thiocytosine nucleoside analogues $[IC_{50} (M)]$

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 NH₃-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 ¹H 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 ([¹⁴C]leucine incorporation) of mouse leukemic L5178Y cells and mouse 3T3 fibroblast were examined as previously described¹ and compared with several known nucleoside analogues (see Table 1). IC₅₀ values are expressed as the molar concentrations leading to a 50% reduction in cell count, colony formation and [¹⁴C]leucine incorporation. The *B*-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

leukemic spleen deoxycytidine kinase					
Compound	Concentration (µM)	Transfer of γ - ³² P from ATP (%)			
dCyd	100	100			
2SdCyd (12)	10	5			
	100	20			
β-2S5FdCyd (14)	10	10			
	100	10			

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

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 μ M and 0.08 μ M, respectively; while 14 was a more potent inhibitor with IC_{50} values of 0.1 μ M and 0.025 μ M, respectively.

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