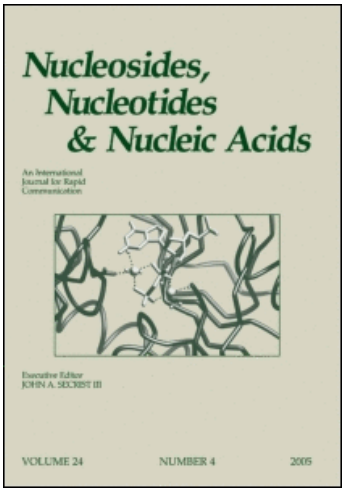

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SYNTHESIS AND ANTITUMOUR PROPERTIES OF 2-THIO-5-CHLORO-NUCLEOSIDES

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Abstract: Four methods are described for the synthesis of 2-thio-5-chlorouracil (1). β- and α-5-Chloro-2-thio-2'-deoxyuridines (12 and 13) were obtained by Lewis acid catalysed condensation of TMS derivative of 1 with 2-deoxy-3,5-di-O-p-toluyl-α-D-ribosyl chloride and deblocking of toluylated derivatives with methanolic ammonia. Selective enzymatic phosphorylation of 12 led to its 5'-monophosphate, the latter being a moderate inhibitor of thymidylate synthase, while 12 showed moderate cytotoxicity *in vitro* against mouse leukemic cells L15178Y.

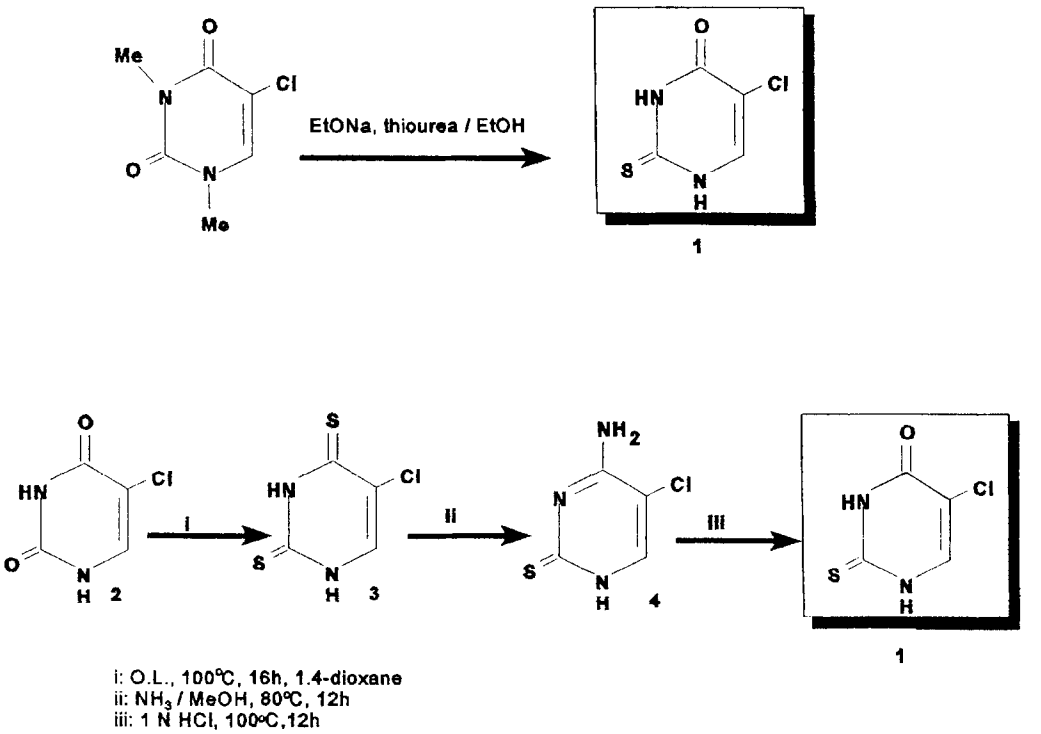
Introduction.

5-Chloro-2'-deoxyuridine exhibits cytotoxicity vs mouse leukemic cells L1210 and is an inhibitor of thymidylate synthase and thymidine kinase.[1,2] This observation, and the fact that substitution of sulphur at the 2-position of uracil leads to an increase in acidity (i.e. decreases the pK_a for dissociation of the proton N-3, which may affect binding of the 2-thionucleosides to the above enzymes) prompted us to undertake its synthesis and to examine the properties of 5-chloro-2-thiouracil nucleosides, as well as the parent base, 5-chloro-2-thiouracil (1).

Chemistry.

Four methods were used for the synthesis of 1: (a) reaction of 5-chloro-1,3-dimethyluracil with thiourea (Scheme 1); (b) deamination of 5-chloro-2-thiocytosine (4) (Scheme 1); (c) chlorination of the p-nitrophenylethyl derivative (6) of 2-thiouracil (Scheme 2); (d) reaction of 2,5-dichloro-4-hydroxypyrimidine (9) with thiourea, which proved the most effective (60% yield) (Scheme 2).

β- and α-5-Chloro-2-thio-2'-deoxyuridines (12 and 13) were obtained by Lewis acid catalysed condensation of TMS derivative of 1 with 2-deoxy-3,5-di-O-p-toluyl-α-D-ribosyl chloride in 1,2-dichloroethane in the presence of $TiCl_4$, to give a mixture of the β- and α-anomers of 2'-deoxy-3',5'-di-O-p-toluyl-5-chloro-2-thiouridine (10 and 11) with a ratio α/β = 1 : 3 (Scheme 3). These were separated by crystallization from methanol and then purified by preparative TLC on silica gel.



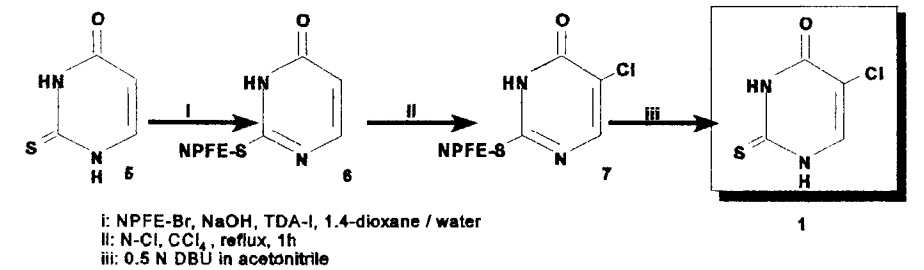
Scheme 1

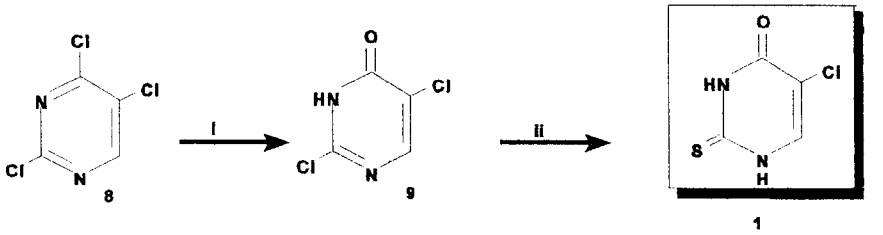
The free 5-chloro-2-thio-2'-deoxyuridine (12) was obtained by deblocking the p-toluyl derivative 10 with a methanolic solution of NH_3. M.p.:180-182°C; UV: λ_{max} (pH 2) 222 nm (ϵ 14300); 277 nm (ϵ 16900) λ_{max} (pH 7) 225 nm (ϵ 11800); 242 nm (ϵ 14000); 274.5 nm (ϵ 17300); λ_{max} (pH 12) 242.5 nm (ϵ 20000); 270.5 nm (ϵ 18600); ^{1}H NMR (500 MHz, D_2O) δ 2.33 (1 H, m, $J_{1',2''}$=6.39 Hz, 2'-H), 2.64 (1 H, m, $J_{1',2''}$=5.17 Hz, 2"-H), 3.83 (1 H, dd, $J_{4',5''}$=4.45 Hz, 5"-H), 3.93 (1 H, dd, $J_{4',5'}$=3.26 Hz, 5'-H), 4.11 (1 H, m, $J_{4',3'}$=4.07 Hz, 4'-H), 4.45 (1 H, m, $J_{2',3'}$=4.54 Hz, $J_{2'',3'}$=6.46 Hz, 3'-H), 6.89 (1 H, br t, 1'-H), 8.39 (1 H, d, 6-H).

The nucleoside 12 was subjected to selective enzymatic phosphorylation with the aid of the wheat shoot nucleoside phosphotransferase system[3] to give 5-chloro-2-thio-dUMP which was quantitatively converted to its parent nucleoside 12 by snake venom 5'-nucleotidase.

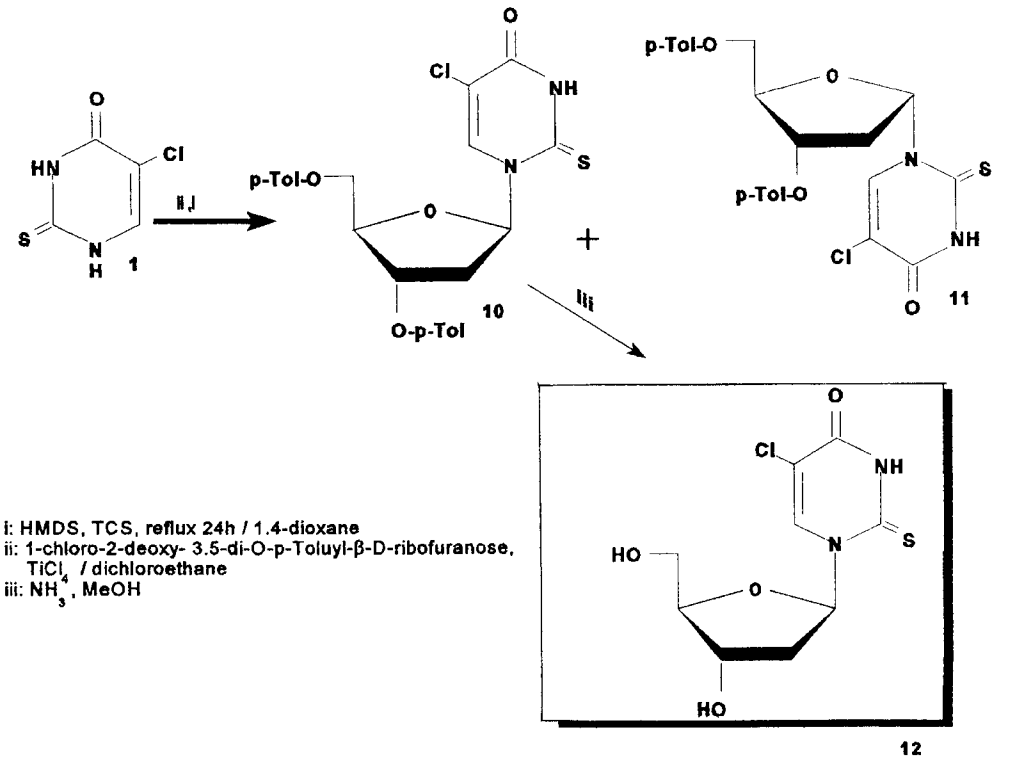
Antitumour Activity and Thymidylate Synthase Inhibition.

The nucleoside 12 was tested for antitumour activity *in vitro* with mouse leukemic cells L15178Y as previously described,[4] and showed weaker cytotoxicity (CD_{50} 8 x 10^{-5} M) than 5-chloro-





Scheme 2



Scheme 3

2'-deoxyuridine (CD_{50} 5 x 10^{-7} M). To explain the mechanism of antitumour activity of 12, the interaction of its 5'-monophosphate with thymidylate synthase was investigated. Preparations of purified Ehrlich ascites carcinoma (EAC) and L1210 thymidylate synthase (TS) were as previously reported.[5] Commercial L. casei TS was purified by affinity chromatography to electrophoretical homogeneity. Enzyme assays and identification of the type of inhibition were as previously described.[6] Spectrophotometric monitoring at 340 nm of the reaction mixture containing 0.30 mM 2-thio-5-chloro-dUMP in place of dUMP, exhibited no substrate activity with L. casei thymidylate synthase. To test whether thymidylate synthase may dehalogenate this nucleoside, absorbance at 280 nm of a mixture 0.30 mM halogenated compound and 5 mM β-mercaptoethanol with TS was recorded. 2-thio-5-chloro-dUMP was not dehalogenated by L. casei enzyme. Garret et al.[7] reported that TS easily dehalogenates BrdUMP and IdUMP but not CldUMP.

Inhibition of Ehrlich ascites carcinoma and L1210 thymidylate synthase by 2-thio-5-chloro-dUMP was tested by varying the dUMP concentration with different concentrations of inhibitor added simultaneously to the reaction mixture. Competitive inhibition, reflected by the intersection at the ordinates of Lineweaver-Burk plots led to apparent K_i values of 6.0 and 23.2 μM for EAC and L1210 TS, respectively. The K_i of L1210 TS for 2-thio-5-chloro-dUMP was over 300-fold higher than that for 2-thio-5-fluoro-dUMP. 2-Thio-5-chloro-dUMP did not cause time-dependent inactivation of thymidylate synthase, while 2-thio-5-fluoro-dUMP is a time-dependent inactivator of TS from many sources.[8]

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