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Synthesis of Some 5'-Thiopentofuranosylpyrimidines as Potential Anti-tumor Agents.

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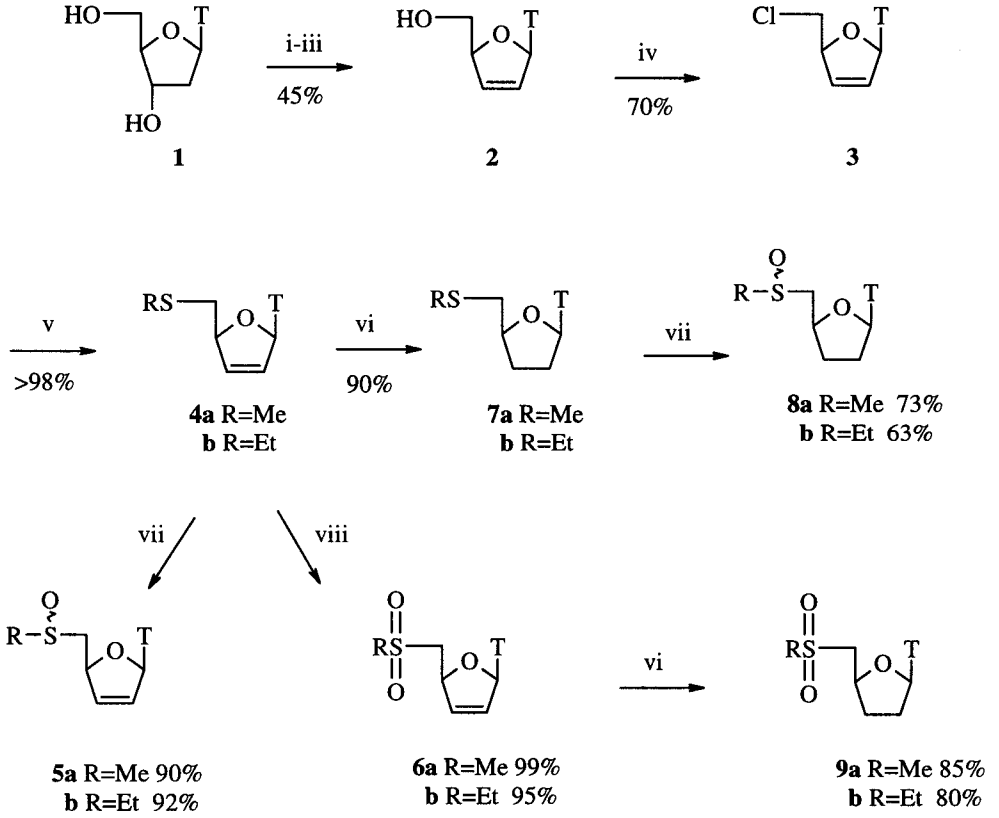
Abstract: The preparation of, hitherto unknown, 2',3'-didehydro-3'-deoxythymidine derived 5'-thioether, sulfoxide, sulfone [**4-9**] is described. The key steps of this synthesis are the nucleophilic displacements of a halogen by a thioalkyl sodium salt, and the later oxidation of the sulfur group into sulfone and sulfoxide analogues. These compounds have been evaluated for their inhibition of L1210 cells proliferation. None of the compounds were active except the 5'-ethylthio analogue **4b** that showed a moderate activity (IC_{50} of 90.2 μM). © 1997 Elsevier Science Ltd.

The thymidine 5'-monophosphate (TMP) which is required for cell proliferation is biosynthesized *via* the *de novo* pathway from deoxyuridine 5'-phosphate. Several drugs are effective in blocking that *de novo* pathway. The alternate route to TMP involves a reaction catalyzed by thymidine kinase. In neoplastic tissue and proliferating cells, the activity of this enzyme is elevated to a high level that permits this enzyme to play a major role in TMP production *in vivo*.[1,2] Evidence indicates a direct correlation between TK content in tumor tissue and tumor growth rate. Two isozymes of TK have been recognized.[3-7] Mitochondrial-thymidine kinase (M-TK) is the predominant form in adult human liver,[4,8] lung,[8] colon,[8] and the cytoplasmic isozyme (C-TK) predominates in human tumor cell lines.[4,9] The data previously reported by Hampton et al.[10,11] suggest that effective neoplastic chemotherapy might be achieved when a drug that blocks *de novo* TMP biosynthesis is coadministrated with a drug that selectively inhibits the C-TK. Several 5'-alkylthionucleosides have been synthesized and 5'-(ethylthio)-5'-deoxythymidine was found to be a noncompetitive inhibitor of the enzyme.[10,11] Recently, other 5'-thioalkyl nucleosides have been reported as antitumor or antiviral agents.[12-14]

As part of our drug discovery program, we initiated the synthesis of 5'-alkylthio thymidines derived as potential anticancer agents. Herein, we wish to report the syntheses of the synthesis and preliminary antitumor evaluation of several, hitherto unknown, 2',3'-didehydro-3'-deoxy-5'-thioether thymidines. The bulk as well as the polar character of the groups attached to the 5'-carbon were varied, as well as the 2',3'-position in the hope that this might bring about an improvement in the therapeutic index based on an increase in substrate specificity

for the C-TK. Finally, we also focused on the synthesis of the 5'-alkylsulfoxide or 5'-alkylsulfone analogues. In fact, during metabolization, organic sulfides can undergo oxidation to sulfoxides, and then to sulfones, whereas sulfoxides but not sulfones can undergo reduction.[15,16] The change in lipophilicity associated with the oxidation state of the S-atom, and body distribution are not straightforward to predict.

Our synthetic strategy to the 5'-alkylthionucleosides utilized the known compound 2',3'-didehydro-3'-deoxythymidine, D4T, **2** as a chiral starting material, which was prepared in three steps from thymidine[17] (Scheme 1).



Scheme 1. Reagents: (i) MsCl, CH_2Cl_2, Et_3N; (ii) NaOH 1N, reflux; (iii) tBuOK, DMSO then toluene; (iv) $SOCl_2$, HMPA; (v) RSH, NaH, THF; (vi) H_2 Pd/C 20 psi; (vii) MMPP 0.5 eq, CH_2Cl_2; (viii) MMPP 1 eq, CH_2Cl_2.

We synthesized the key intermediate 5'-chloro analogue **3** by addition of $SOCl_2$ in HMPA to **2**. Treatment of **3** at low temperature by an excess of sodium methylthioate or ethylthioate gave respectively the 5'-*S*-methyl-5'-thio-D4T **4a** and 5'-S-ethyl-5'-thio analogue **4b** with quantitative yield. The oxidation of **4a** and **4b** with 0.5 eq of MMPP gave the diastereomeric sulfoxides, 5'-methylsulfinyl-($S_{R/S}$)-D4T **5a** (90%) and

5'-ethylsulfinyl-($S_{R/S}$)-D4T **5b** (92%) respectively. The oxidation of **4a** and **4b** with one equivalent of monoperoxyphtalic acid (MMPP) gave the sulfone derivatives **6a** (99%) and **6b** (95%).[18] The hydrogenation of **4a** and **4b** yielded the 5'-thioether-2'-deoxythymidine **7a** and **7b** respectively with quantitative yield.[19] It is interesting to note that the sulfur did not decrease the activity of the Pd/C used as catalyst for the hydrogenation. Oxidation of **7a** and **7b** gave a diastereomeric mixture of sulfoxides **8a** (73%) and **8b** (63%) respectively.[20] The sulfone analogues **9a** (85%) and **9b**[21] (80%) were obtained directly by the hydrogenation under Pd/C of the D4T-derivatived 5'-sulfone **6a** and **6b**.[22] The anti-cancer activity for the synthesized compounds was evaluated. However, none of the compounds did show any significant anti-cancer activity (IC_{50} > 100μM) on the L1210 cells[23] except 3'-deoxy-2',3'-didehydro-5'-ethylthio thymidine that exhibited a moderate activity (IC_{50} = 90.2 μM). Other biological evaluations are in progress.

In summary, the synthesis of hitherto unknown 2',3'-didehydro-3'-deoxythymidine derived 5'-alkyl-sulfides, sulfones and, sulfoxides has been accomplished. Synthesis of other 5'-alkylthio pyrimidine and purine nucleosides is in progress in our laboratory.

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18 General procedure for the oxidation : anhydrous MMPP (0.5 eq. to afford the sulfoxide derivatives or 1 eq. to the sulfone derivatives), previously dissolved in CH_2Cl_2 were added to a solution of 5'-S-alkyl-5'-thionucleosides (**4a,b** or **7a,b**) (1 mmol) in CH_2Cl_2/MeOH (9:1, v/v, 30 mL). The reaction was stirred with gentle reflux for 2 h. Then, the mixture was cooled, filtered and evaporated to dryness without heating, under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 9:1, v/v) to give a white solid.

19. Selected spectroscopic data for thioether-**7b** : mp.83-86 °C; *^{1}H NMR δ ($CDCl_3$) 8.50 (br s, 1H, NH), 7.46 (s, 1H, H_6), 6.06 (dd, 1H, J = 6.45, 3.75Hz, $H_{1'}$), 4.21 (m, 1H, $H_{4'}$), 2.85 (d, 2H, $H_{5',5''}$), 2.60 (q, 2H, J = 7.33 Hz, CH_2-S), 2.11 (m, 4H, $H_{2'}$, $H_{2''}$, $H_{3'}$, $H_{3''}$), 1.91 (s, 3H, Me), 1.25 (t, 3H, J = 7.32 Hz, $\underline{CH_3}$-CH_2S-); Anal. ($C_{12}H_{18}N_2O_3S$) calcd: C 53.33, H 6.66, N 10.37, S 11.85; Found: C 53.35, H 6.95, N 10.25, S 11.81.*

20. Selected spectroscopic data for sulfoxide-**5'-S(R/S)-8b** : mp. 161-163 °C; *^{1}H NMR δ (MeOD) 7.54 (s, 1H, H_6), 6.09 (m, 1H, $H_{1'}$), 4.34 (m, 1H, $H_{4'}$), 3.20 (m, 2H, $H_{5',5''}$), 2.93 (m, 2H, CH_2-S(O)-), 2.45 (m, 1H, $H_{2'}$), 2.33-1.95 (m, 3H, $H_{2''}$, $H_{3'}$, $H_{3''}$), 1.91 (s, 3H, Me), 1.36 (dd, J = 7.34, 1.96 Hz, $\underline{CH_3}$-CH_2S(O)-); Anal. ($C_{12}H_{18}N_2O_4S$.0.1% H_2O) calcd: C 50.88, H 6.37, N 9.72; Found: C 50.33, H 6.25, N 10.20.*

21. Selected spectroscopic data for sulfone **9b** : mp. 201-202 °C; *^{1}H NMR δ (DMSO-d6) 11.26 (s, 1H, NH), 7.54 (d, 1H, J = 1.23Hz, H_6), 5.99 (m, 1H, $H_{1'}$), 4.29 (m, 1H, $H_{4'}$), 3.71 (dd, 1H,J = 14.67 Hz, 8.3 1Hz, $H_{5'}$), 3.41 (dd, 1H, J = 14.67 Hz, 4.40 Hz, $H_{5''}$), 3.05 (m, 2H, CH_2-SO_2), 2.18 (m, 2H, $H_{2',2''}$), 1.91 (m, 2H, $H_{3',3''}$), 1.77 (d, 3H, J = 1.23Hz, 5-Me), 1.16 (t, 3H, J = 7.38 Hz, $\underline{CH_3}$-CH_2SO_2); Anal. ($C_{12}H_{18}N_2O_5S$) calcd: C 47.67, H 6.00, N 9.27; Found: C 47.63, H 5.92, N 9.21.*

22. All key intermediates and final compounds **3-9** in Scheme I gave correct elemental analyses (± 0.5%). These new compounds **3-9** were purified by column chromatography and product structures were determined by infrared, MS, 250 MHz ^{1}H NMR and ^{13}C NMR.

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