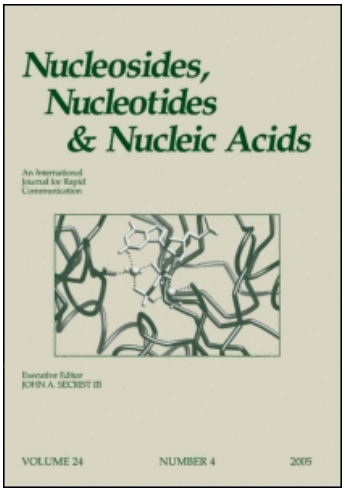

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Felczak, K. , Kulikowski, T. , Vilpo, J. A. , Giziewicz, J. and Shugar, D.(1987) 'Synthesis and Biological Properties of 6-Substituted 5-Fluorouridines', Nucleosides, Nucleotides and Nucleic Acids, 6: 1, 257 — 260
To link to this Article: DOI: 10.1080/07328318708056199
URL: http://dx.doi.org/10.1080/07328318708056199

NUCLEOSIDES & NUCLEOTIDES, 6(1&2), 257-260 (1987)

SYNTHESIS AND BIOLOGICAL PROPERTIES OF 6-SUBSTITUTED 5-FLUOROURIDINES

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Summary: Procedures are described for the synthesis of 5-fluoro-6-methyluridine (9) and 5-fluoro-6-fluoromethyl-uridine (11), both in the fixed syn conformation, and their in vitro antitumour activities. In particular, 11 was found to be a potent antitumour agent.

Nucleosides of 5-fluorouracil (FUra), viz. 5-fluoro-uridine (FUrd) and 5-fluoro-2'-deoxyuridine (FdUrd) exhibit potent antitumour activities, but are rapidly degraded in vivo by uridine and thymidine phosphorylases.[1-3] It was previously shown that 6-methyluridine, a nucleoside constrained to the syn conformation by the bulky 6-methyl substituent,[4,5] is a reasonably good substrate for uridine phosphorylase; whereas the reverse, synthetic, reaction with 6-methyluracil (1) as substrate does not occur.[6]

It therefore appeared of interest to prepare 5-fluoro-6-methyluracil (2a) and 5-fluoro-6-methyluridine (9), and to examine their substrate properties vs uridine phosphorylase and their antitumour activities. Since published physico-chemical data for 2a are conflicting,[7,8] and reported data[8] for 9 are incomplete and raise doubts as to the presence of a 5-fluoro substituent, it was decided to prepare 2a and 9 by two new procedures (Fig. 1): (a) fluorination

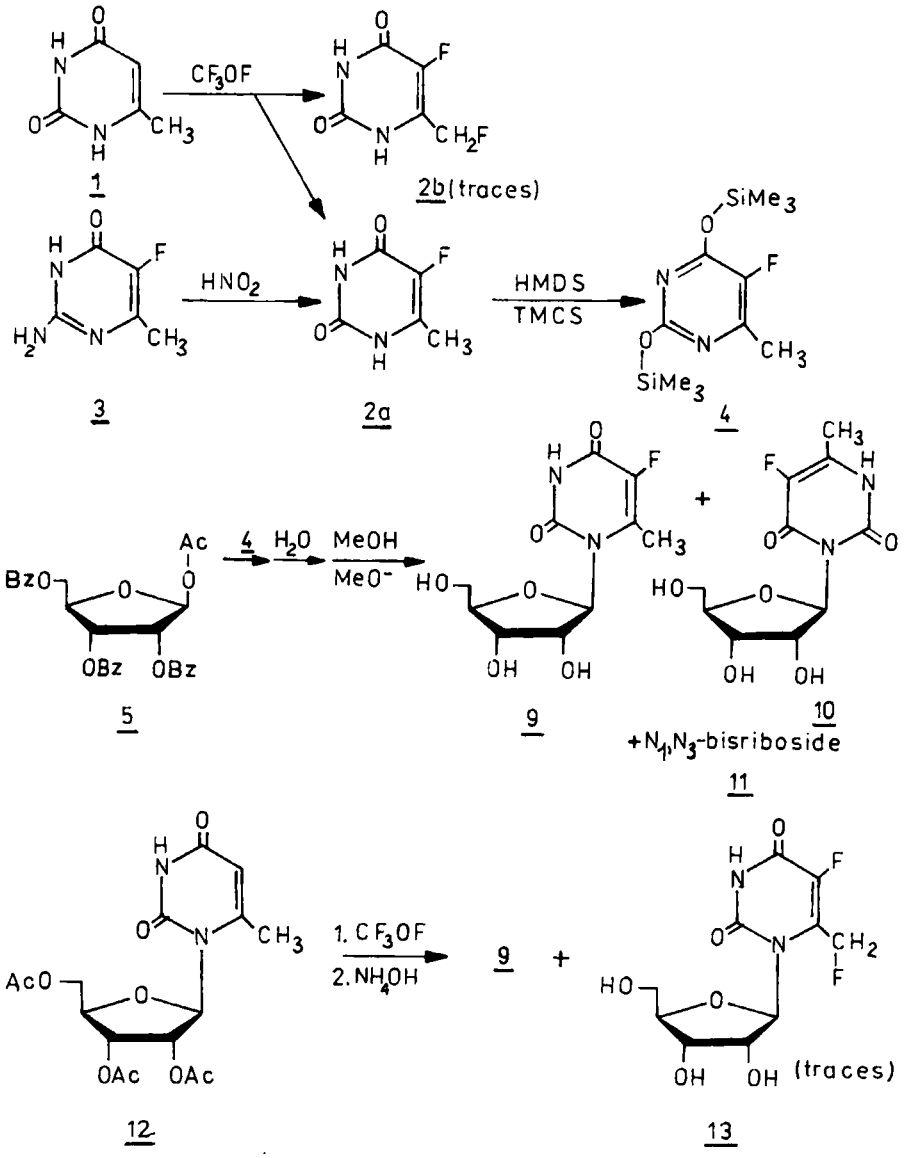

Fig. 1.

of 1 with CF_3OF in $CFCl_3$ at -78°C,[9] which gave 2a in 93% yield, m.p. 307-310°C (decomp.); $\lambda_{max}^{pH\ 2}$ 269 nm (ε_{max} 7.0 × 10^3); $\lambda_{max}^{pH\ 14}$ 286 nm (ε_{max} 6.9 × 10^3); pK_a 7.7; ^1H NMR (DMSO-d_6) δ(ppm vs internal TMS) 11.37 (1H, bs, H-1), 10.79 (1H, bs, H-3), 2.06 (3H, m, $^4J_{H-F}$ 5.2 Hz, 6-CH_3); MS m/e 144.2 $|M^+|$ and a very faint peak (M^+ 162.2) corresponding to 2b (see below); (b) deamination of 5-fluoro-6-methylisocytosine (3) with $NaNO_2/CH_3COOH$, which gave 2a quantitatively, with properties identical to the obtained by method (a).

Two different procedures were also applied to synthesis of 9 : (a) the triflate-catalyzed condensation of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (5) with 2,4-bis-O-(trimethylsilyl)-5-fluoro-6-metyluracil (4) in CH_3CN. The resulting products, following desilylation with $HOH/NaHCO_3$, then debenzoylation in CH_3ONa/MeOH, yielded 9, its N(3)-isomer (10), and the N(1), N(3)-diriboside (11) in the ratio 2:1:1; (b) fluorination of 2',3',5'-tri-O-acetyl-6- methyluridine (6) with CF_3OF in $CHCl_3$, followed by deacetylation with NH_4OH.

The product obtained by method (b) exhibited unusually high cytotoxicity relative to that obtained by method (a). TLC on silica gel with EtOAc:PrOH:H_2O (4:2:1 , v/v) demonstrated the presence of two components (R_f 0.53, m.p. 180°C and R_f 0.63, m.p. 184°C) in the ratio 16:1. The slower migrating product was identified as 9, identical with that from method (a): $\lambda_{max}^{pH\ 2}$ 269 nm (ε_{max} 9.5 × 10^3), λ_{max}^{pH12} 269 nm (ε_{max} 7.3 × 10^3); pK_a 7.7; ^1H NMR, 5.59 (1H, d, $J_{1',-2'}$, 3.3 Hz, $H_{1'}$), 4.82 (1H, m, $J_{2',-3'}$, 6.3 Hz, $H_{2'}$), 4.39 (1H, t, $J_{3',-4'}$, 7.4 Hz, $H_{3'}$), 3.97 (1H, m, $J_{4',-5'}$, 2.8 Hz, $J_{4',-5''}$, 6.3 Hz, $H_{4'}$), 3.90 (1H, m, $J_{5',-5''}$, -12.3 Hz, $H_{5'}$), 3.75 (1H, m, $H_{5''}$), 2.21 (2H, d, $^4J_{F-H}$ 3.8 Hz, 6-CH_3); MS m/e 276 $|M^+|$.

The structure of the more rapidly migrating product was also established by UV, MS and ^1H NMR spectroscopy as 5-fluoro-6-fluoromethyluridine (13), with a pK_a for dissociation of the N(3)-H of 7.1 as compared to 7.70 for 9 and 7.75 for FUrd[10].

Both 9 and 13 were readily phosphorylysed by E. coli uridine phosphorylase to release 2a, identical with a synthetic sample and with literature data,[7] and 2b, m.p. 240°-242°C the structure of which was conformed by UV, MS and ^{1}H NMR: 11.67 (1H, bs, H-1), 11.43 (1H, bs, H-3), 5.25 (2H, dd, $^2J_{F-H}$ 49.8 Hz, $^4J_{F-H}$ 2.8 Hz, 6-CH_2F).

Cytotoxicities of FUra, 2a, 2b, and their nucleosides were tested vs 7 human tumour cell lines, PHA-stimulated human lymphocytes, and mouse L1210 cells by procedures described elsewhere. Particularly noteworthy was the finding that the activity of the difluoro nucleoside 13 against most of the cell lines was of the same order of magnitude as that of FdUrd. The monofluoro nucleoside 9, and the bases 2a and 2b exhibited only marginal activity.

Details of syntheses, conformational analyses, and tests of cytotoxicities, are in preparation.

ACKNOWLEDGMENTS: We are indebted to Dr. Alicja Drabikowska for enzymatic tests, and to Dr. Ryszard Stolarski for help with the NMR spectra. This investigation was supported by the Polish Cancer Research Program (CPBR 11.5-109).

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