Electrophilic Substitution in Dihydrouracils

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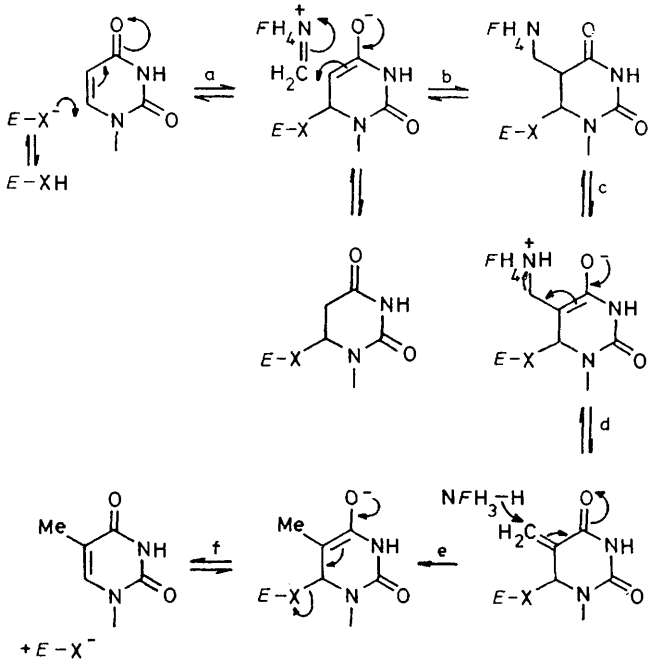
Summary 2′-Deoxyuridine photohydrate with dimethylamine and formaldehyde forms 5-dimethylaminomethyl-2′-deoxyuridine in a very fast reaction the mechanism of which bears on thymidylate synthetase catalysis.

THYMIDYLATE SYNTHETASE catalyses the reductive methylation of deoxyuridylate as the last step in the biosynthesis of thymidylic acid.[1] At present it is held that the initial alkylation by the co-factor 5,10-methylenetetrahydrofolate ($FH_4N^+{=}CH_2$) is as shown in the Scheme; carbanion reactivity at C-5 is generated by addition of a nucleophilic residue (*E*–XH) in the protein (step a), followed by Mannich condensation with the methylene group of the co-factor (step b). Reduction is considered to result from anion formation (step c), exocyclic elimination of tetrahydrofolate (FH_4N) (step d), and hydride transfer from the latter (step e). The process is then completed by elimination of *E*–XH, releasing thymidylate (step f).[2,3]

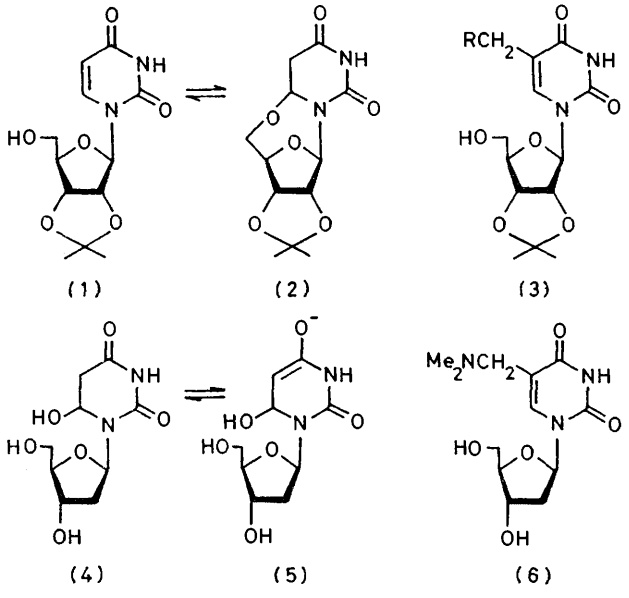
In support of this scheme there is much evidence to show that reversible addition of nucleophiles at C-6 in uracils catalyses proton exchange at C-5.[4] Moreover the transient formation from, for example, 2′,3′-*O*-isopropylideneuridine (**1**) of the cyclonucleoside (**2**) in base accounts for the rapid proton exchange at C-5 and, in the presence of formaldehyde, of 5-hydroxymethylation (**3**; R = OH).[2]

In a reaction more closely analogous to that catalysed by thymidylate synthetase, and similar to the one observed with uridine under more vigorous conditions,[5] we find that (**1**) with dimethylamine and formaldehyde at pH 10·5 gives 86% of (**3**; R = NMe_2) in 70 h at room temperature. The methiodide (**3**; R = NMe_3^+) is reduced rapidly and completely by aqueous alkaline borohydride to 5-methyl-isopropylideneuridine (**3**; R = H). The reduction evidently proceeds through a C-6 adduct by an exocyclic elimination–addition mechanism (as in steps d and e in the Scheme) since (**3**; R = NMe_3^+) also undergoes a fast exchange in methanolic methoxide to give the 5-methoxymethyl derivative (**3**; R = OMe).



SCHEME

While the kinetically-identified intermediate (**2**) is clearly involved in these electrophilic substitution reactions at C-5[2] it is noteworthy that 1-methyl-5,6-dihydrouracil undergoes proton exchange at C-5 only very slowly.[6] It follows that substitution at C-6 influences ionisation at C-5; convenient models to investigate this view can be obtained by photoaddition to uracil derivatives. 2′-Deoxyuridine, on irradiation in water gives the hydrate (**4**).[7] When a dilute solution of (**4**) (1 g l^{-1}) was treated with a 10 molar excess of dimethylamine and formaldehyde at pH 10·0 for 20 min (considerably longer than required for the absorbance at 263 nm to attain its maximum value), 5-dimethylaminomethyl-2′-deoxyuridine (**6**) was isolated (74% based on an 80% formation of the photohydrate). Diethylamine and pyrrolidine in place of dimethylamine gave the corresponding 5-substituted products (59 and 98% yields, respectively). 2′-Deoxyuridine, itself, does not undergo reaction under these conditions.



We conclude that the success of the reaction demonstrates that the acidic dissociation to give (**5**) is very greatly enhanced by the electronegative substituent at C-6 and that this is a relevant but hitherto unrecognised factor in thymidylate synthetase catalysis.

The extent to which the substitution reaction competes with irreversible elimination of water in (**4**) is also interesting and suggests this as a potentially general approach to carbon–carbon bond formation at C-5. We hope to extend its scope with other electrophiles and other, more synthetically versatile uracils.

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