

## Electrophilic Substitution in Dihydrouracils

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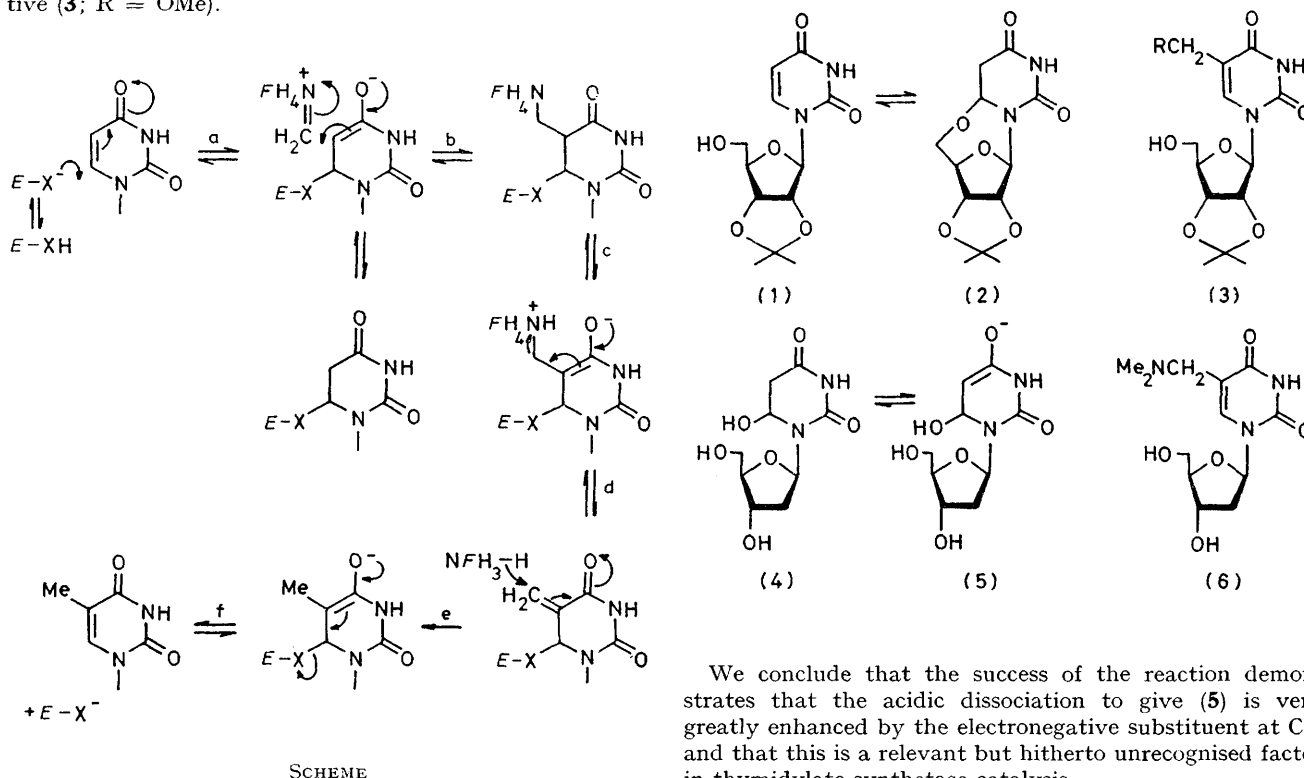
**Summary** 2'-Deoxyuridine photohydrate with dimethylamine and formaldehyde forms 5-dimethylaminomethyl-2'-deoxyuridine in a very fast reaction the mechanism of which bears on thymidylate synthetase catalysis.

THYMIDYLATE SYNTHETASE catalyses the reductive methylation of deoxyuridylate as the last step in the biosynthesis of thymidylic acid.<sup>1</sup> 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).<sup>2,3</sup>

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.<sup>4</sup> 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).<sup>2</sup>

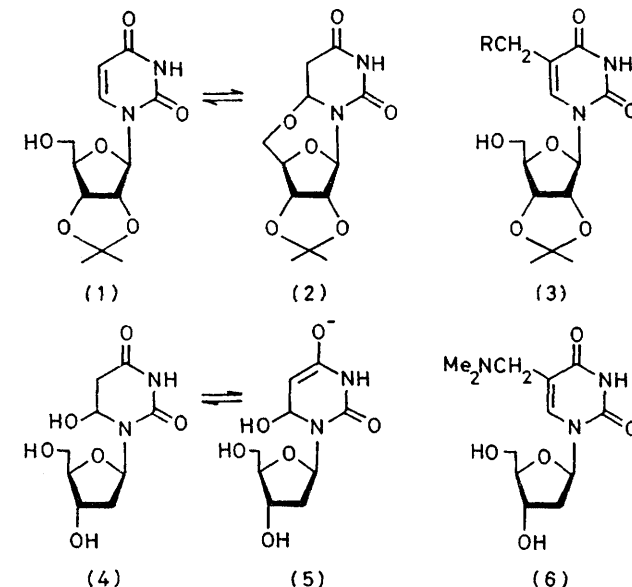
In a reaction more closely analogous to that catalysed by thymidylate synthetase, and similar to the one observed with uridine under more vigorous conditions,<sup>5</sup> we find that (1) with dimethylamine and formaldehyde at pH 10.5 gives 86% of (3; R = NMe<sub>2</sub>) in 70 h at room temperature. The methiodide (3; R = NMe<sub>3</sub><sup>+</sup>) is reduced rapidly and completely by aqueous alkaline borohydride to 5-methylisopropylideneuridine (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<sub>3</sub><sup>+</sup>) 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<sup>2</sup> it is noteworthy that 1-methyl-5,6-dihydrouracil undergoes proton exchange at C-5 only very slowly.<sup>6</sup> 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).<sup>7</sup> When a dilute solution of (4) (1 g l<sup>-1</sup>) 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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<sup>1</sup> S. J. Benkovic and W. P. Bullard, 'Progress in Bioorganic Chemistry,' eds. E. T. Kaiser and F. J. Kezdy, Wiley-Interscience, New York, 1973, vol. 2, p. 133.

<sup>2</sup> D. V. Santi and C. F. Brewer, *Biochemistry*, 1973, **12**, 2416.

<sup>3</sup> A. L. Pogolotti and D. V. Santi, *Biochemistry*, 1974, **13**, 456.

<sup>4</sup> See *inter alia*: H. Hayatsu, *Progr. Nucleic Acid Res. Mol. Biol.*, 1976, **16**, 75.

<sup>5</sup> N. K. Kochetkov, E. I. Budowsky, V. N. Shibaev, and G. I. Eliseeva, *Izvest. Akad. Nauk. S.S.S.R., Ser. khim.*, 1966, 1779 (*Chem. Abs.*, 1967, **66**, 105151r).

<sup>6</sup> D. M. Brown and P. F. Coe, *Chem. Comm.*, 1970, 864.

<sup>7</sup> I. Pietrzykowska and D. Shugar, *Acta Biochim. Pol.*, 1974, **21**, 187; *Biochem. Biophys. Res. Comm.*, 1969, **37**, 225; J. Cadet, L-S. Kan, and S. Y. Wang, *J. Amer. Chem. Soc.*, 1978, **100**, 6715.