5-Substituted Pyrimidine Nucleosides and Nucleotides

By T. K. Bradshaw and D. W. Hutchinson
DEPARTMENT OF MOLECULAR SCIENCES, UNIVERSITY OF WARWICK, COVENTRY CV4 7AL

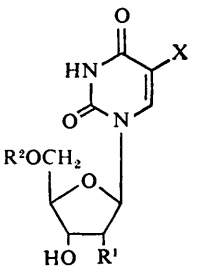
1 Introduction

Pyrimidine nucleosides and nucleotides bearing substituents other than hydrogen or methyl in the 5-position of the heterocyclic ring are analogues of natural components of nucleic acids and coenzymes. Many methods have been developed for their synthesis and the biological properties of these analogues have been widely studied. Polynucleotides containing 5-substituted pyrimidines have also been prepared and have been used to obtain information on the physical chemistry of polynucleotides.

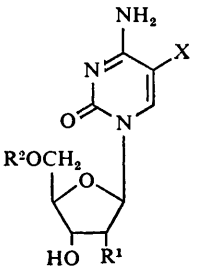
Several extensive monographs have been published on the chemistry of nucleosides and nucleotides in general[1] but none expands upon the particular area of synthesis and modification of 5-substituted pyrimidine nucleosides and nucleotides. N(1)-Substitution of the pyrimidine ring can have a profound effect on its reactivity at the 5-position and this has been the cause of many conflicting reports which have appeared in the literature on the synthesis and modification of 5-substituted pyrimidines. For example, model reactions carried out on pyrimidine bases are frequently not applicable to nucleosides and nucleotides, while the nucleosides and nucleotides themselves often differ in their reactivity towards electrophiles.

In this review, we shall consider the published data on the chemical synthesis and reactions of these compounds, in particular of uridine (1; X = H, R^1 = OH, R^2 = H) and cytidine (2; X = H, R^1 = OH, R^2 = H) derivatives. We will attempt to rationalize data by proposing a limited number of pathways which a reaction might follow and will interpret a number of biochemical reactions in the light of these proposals. In view of the often incomplete information on experimental conditions in the literature and in the absence of a rigorous structure proof for some reaction products, it is often only possible to speculate on a reaction pathway. However, we feel that these speculations may be of value to those who intend to synthesize other new 5-substituted pyrimidine nucleosides and nucleotides.

[1] (*a*) A. M. Michelson, 'The Chemistry of Nucleosides and Nucleotides', Academic Press New York, 1963; (*b*) 'Basic Principles in Nucleic Acid Chemistry', ed. P. O. P. T'so Academic Press, New York, 1974, Vols. 1 and 2; (*c*) 'Organic Chemistry of Nucleic Acids', ed. N. K. Kochetkov and E. I. Budovskii, Plenum Press, London and New York, 1972 Vols. 1 and 2.



(1)

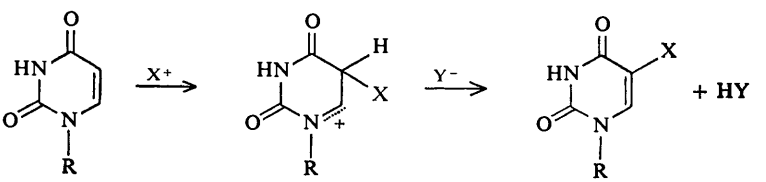


(2)

2 Substitution at C(5) in the Pyrimidine Ring

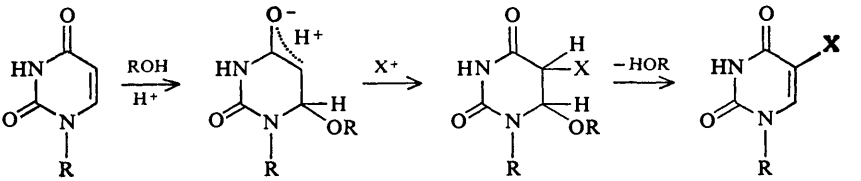
A. Reaction Mechanisms.—As will be discussed below, the complex nature of the products which can be obtained under conditions when 5-substitution of pyrimidine nucleosides and nucleotides takes place makes mechanistic interpretation of reaction pathways difficult and little has been published on this topic. For example the nature of the solvent, substitution on the sugar moiety, *etc.* play important parts in these reactions. However, we propose that three types of mechanism can be invoked to explain direct substitution reactions.

Type 1. In some situations (Scheme 1), the pyrimidine bases exhibit aromatic properties, and reaction at the electronegative C(5) of the heterocyclic ring could proceed by a mechanism analogous to that for electrophilic aromatic substitution involving a sigma complex which would be stabilized by election donation from the adjacent nitrogen atom. Loss of a proton from the intermediate would give the 5-substituted pyrimidine derivative. The same intermediate would arise if Markovnikov addition of the electrophile to the 5,6-double bond of the pyrimidine occurred, and it is difficult from the published data to distinguish between these two possibilities. Cytidine derivatives frequently fail to take part in Type 1 reactions under conditions in which uridine derivatives react readily. One reason for this difference may be the basic nature of the cytidine ring. If the cytidine ring acquires a positive charge by reacting on nitrogen with an electrophile, then further reaction at C(5) would be hindered.



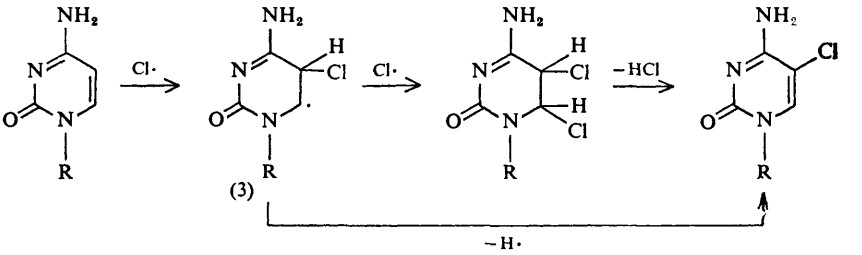
Scheme 1

Type 2. In the second mechanism (Scheme 2), nucleophilic addition at C(6) occurs before electrophilic attack at C(5) and the reaction pathway resembles the well-known Michael reaction. The nucleophile which attacks C(6) can be, for example, water, an alcohol (particularly the 5′-hydroxyl of the sugar residue in a nucleoside), or halide ion. A necessary consequence of this reaction pathway is that the nucleophile is later eliminated with the proton at C(5) to regenerate the 5,6-double bond and it is reasonable to assume that the nucleophile and the hydrogen at C(5) must be *trans* to one another for ready elimination to occur. The stereochemistry of the Michael reaction is complex and both *cis*- and *trans*-addition has been observed.[2] If initial *cis*-addition occurs then the final elimination can take place without difficulty. If a *trans*-addition to uridine or cytidine occurs, as has been observed with thymidine,[3] then epimerization at C(5) must take place before the final *trans*-elimination can occur; such epimerizations have been observed.[3b] Many of these reactions occur in the presence of acid and protonation of the pyrimidine ring might be expected to assist nucleophilic attack at C(6) as well as the elimination of the nucleophile in the last stage.



Scheme 2

Type 3. A third reaction pathway (Scheme 3) can be envisaged involving free radicals. The photochemical dimerization, hydration, and addition of thiols to cytosine, uracil, and their nucleosides or nucleotides are well known.[4] Pre-

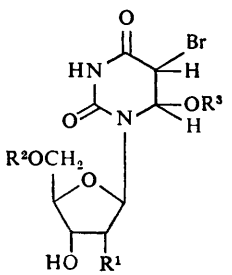


Scheme 3

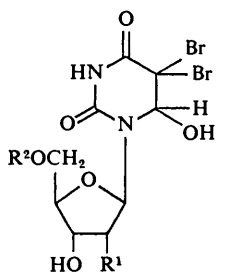
[2] H. O. House, 'Modern Synthetic Reactions', W. A. Benjamin Inc., Menlo Park, California, 1972, 2nd Edn, p. 615.
[3] (*a*) R. T. Teoule, B. Fouque, and J. Cadet, *Nucleic Acid Res.*, 1975, **2**, 487; (*b*) D. Lipkin and J. A. Rabi, *J. Amer. Chem. Soc.*, 1971, **93**, 3309.
[4] (*a*) J. G. Burr, *Adv. Photochem.*, 1968, **6**, 193; (*b*) N. C. Yang, R. Okazaki, and F. Liu, *J.C.S. Chem. Comm.*, 1974, 462.

sumably, addition of free radicals to the excited pyrimidine nucleus takes place, giving rise to intermediates such as (3). Loss of a hydrogen radical from (3) would lead directly to a 5-substituted pyrimidine or alternatively a second free radical could add to (3) to give a dihydropyrimidine. Elimination reactions such as described in the Type 2 mechanism above would then be necessary to regenerate a 5-substituted pyrimidine.

B. Halogenation.—The direct halogenation of pyrimidine bases, nucleosides, and nucleotides has been well studied as these derivatives have important biological properties, *e.g.* as antiviral agents. All three types of mechanism have been suggested for halogenation reactions and all probably occur under different conditions. Uridine[5] and 2′-deoxyuridine[6] react with chlorine in glacial acetic acid to give the chlorinated nucleosides with fully acetylated sugar residues and the same conditions can be used for the chlorination of uridine 2′(3′)-[7] and 5′-phosphates[8] although the sugar residues are not acetylated in these instances. This reaction probably occurs by a Type 1 mechanism and this may be general for other halogenation reactions in non-aqueous solvents, *e.g.* the bromination of uridine in acetic anhydride[9] or *NN*-dimethylformamide.[10] In aqueous[5b,11,12] or alcoholic solution[13] a Type 2 mechanism appears to prevail with the addition of a hydroxylic function (*e.g.* the 5′-hydroxyl of the sugar, water, or alcohol solvent) to the 6-position followed by addition of a bromium ion at C(5). Treatment of the adduct, *e.g.* 5-bromo-6-hydroxy-5,6-dihydrouridine



(4)



(5)

[5] (*a*) D. Visser, K. Dittmer, and I. Goodman, *J. Biol. Chem.*, 1947, **171**, 377; (*b*) T. K. Fukuhara and D. W. Visser, *J. Biol. Chem.*, 1951, **190**, 95.
[6] D. W. Visser, D. M. Frisch, and B. Huang, *Biochem. Pharmacol.*, 1960, **5**, 157.
[7] R. Letters and A. M. Michelson, *J. Chem. Soc.*, 1962, 71.
[8] A. M. Michelson, J. Dondon, and M. Grunberg-Manago, *Biochim. Biophys. Acta*, 1962, **55**, 529.
[9] D. W. Visser in 'Synthetic Procedures in Nucleic Acid Chemistry', ed. W. W. Zorbach and R. S. Tipson, Wiley, New York, 1968, Vol. 1, p. 409.
[10] J. Duval and J. P. Ebel, *Bull. Soc. Chim. biol.*, 1964, **46**, 1059.
[11] P. A. Levene and F. B. La Forge, *Chem. Ber.*, 1912, **45**, 608.
[12] R. E. Beltz and D. W. Visser, *J. Amer. Chem. Soc.*, 1955, **77**, 736.
[13] S. Y. Wang, *Photochem. and Photobiol.*, 1962, **1**, 37.

(4; R^1 = OH, R^2 = H, R^3 = H), with acid leads to 5-bromouridine (1; X = Br, R^1 = OH, R^2 = H). If excess bromine is present, a 5,5-dihalogenouridine (5; R^1 = OH, R^2 = H) can be formed which loses hypobromous acid to form 5-bromouridine. Direct iodination of uridine,[14] 2′-deoxyuridine,[15] and their nucleotides[7] occurs in the presence of aqueous nitric acid. 'Iodine nitrate' has been suggested as the iodinating agent and these reactions probably take place by a Type 1 mechanism.

Treatment of uridine 5′-phosphate or 5′-diphosphate with aqueous bromine does not give the 5-bromo- but rather the 5-hydroxy-nucleotide (1; X = OH, R^1 = OH, R^2 = H_2PO_3 or $H_3P_2O_6$).[16] In this case the ribose ring does not have a free 5′-hydroxy-group which can add on to C(6) of the pyrimidine, and the uridine bromohydrin (4; R^1 = OH, R^2 = H_2PO_3, R^3 = H) may be formed instead. Displacement of bromide from (4; R^1 = OH, R^2 = H_2PO_3, R^3 = H) by water would give a 5,6-dihydro-5,6-dihydroxyuridine. The 5-proton of the latter is easily lost leading to elimination of water and regeneration of the 5,6-double bond in the 5-hydroxyuridine nucleotides. 5-Bromouridine nucleotides can be prepared from the unsubstituted nucleotide and bromine[17] or *N*-bromosuccinimide[18,19] in formamide, and from bromine in aqueous solution when nitric acid is present,[7] when a Type 1 pathway may be followed. The most convenient way of preparing 5-bromo-UMP, however, is by phosphorylation of 2′,3′-*O*-isopropylidene-5-bromouridine (6; X = Br, R = H).[16a]

The fluorination of fully acetylated uridine ribo- and deoxyribo-nucleosides with trifluoromethyl hypofluorite[20] in an inert solvent is an example of a reaction which may occur by either a Type 1 or a Type 2 pathway. In the latter case, *trans*-addition across the 5,6-double bond followed by elimination of trifluoromethanol would be a likely reaction scheme. The fluorination of cytidine has also been achieved with trifluoromethyl hypofluorite.[21]

The chlorination of cytidine in glacial acetic acid does not yield 5-chlorocytidine under conditions in which uridine is successfully chlorinated. Chlorination of cytidine only occurs after irradiation with ultraviolet light,[22] when a Type 3 mechanism is the most likely. Similarly the bromination of cytidine and deoxycytidine can be achieved by ultraviolet irradiation.[23] Although the bromination of deoxycytidine will occur in the absence of light,[24] addition of aqueous

[14] W. H. Prusoff, W. L. Holmes, and A. D. Welch, *Cancer Res.*, 1953, **13**, 221.
[15] (*a*) W. H. Prusoff, *Biochim. Biophys. Acta*, 1959, **32**, 295; (*b*) D. J. Silvester and N. D. White, *Nature*, 1963, **200**, 65.
[16] (*a*) T. Ueda, *Chem. and Pharm. Bull. (Japan)*, 1960, **8**, 455; (*b*) D. W. Visser and P. Roy-Burman in 'Synthetic Procedures in Nucleic Acid Chemistry', ed. W. W. Zorbach and R. S. Tipson, Wiley, New York, 1968, Vol. 1, p. 493.
[17] M. J. Bessman, I. R. Lehman, J. Adler, S. B. Zimmerman, E. S. Sims, and A. Kornberg, *Proc. Nat. Acad. Sci. U.S.A.*, 1958, **44**, 633.
[18] A. M. Michelson, *J. Chem. Soc.*, 1958, 1957.
[19] J. Smrt and F. Šorm, *Coll. Czech. Chem. Comm.*, 1960, **25**, 553.
[20] M. J. Robins and S. R. Naik, *J. Amer. Chem. Soc.*, 1971, **93**, 5277.
[21] J. O. Folayan and D. W. Hutchinson, *Biochim. Biophys. Acta*, 1974, **340**, 194.
[22] T. K. Fukuhara and D. W. Visser, *J. Amer. Chem. Soc.*, 1955, **77**, 2393.
[23] D. M. Frisch and D. W. Visser, *J. Amer. Chem. Soc.*, 1959, **81**, 1756.
[24] (*a*) P. K. Chang and A. D. Welch, *Biochem. Pharmacol.*, 1961, **6**, 50; (*b*) P. C. Srivastava and K. L. Nagpal, *Experientia*, 1970, **26**, 220.

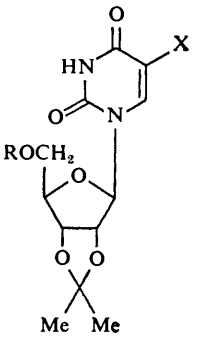
bromine to cytidine gives 5-bromo-6-hydroxy-5,6-dihydrocytidine in analogy to the reaction with uridine.[22] All attempts to prepare 5-bromocytidine from this intermediate were, however, unsuccessful.

Cytidine nucleotides are readily halogenated in aqueous solution,[25,26] formamide,[27] DMF,[28] or acetic acid.[29] The presence of acidic phosphoryl groups on the 5′-hydroxyl prevents the involvement of this hydroxy-group in the addition reaction and hence the reaction pathway is probably Type 1. The iodination of cytidine and its nucleotides by iodine and iodic acid in glacial acetic acid[30] or by iodine and iodine trichloride in nitric acid[31] can also be explained by a Type 1 mechanism.

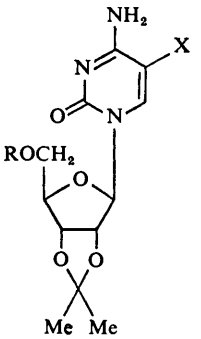
C. Hydroxymethylation.—Nowhere is the difference in reactivity of the various uridine and cytidine derivatives more clearly demonstrated than in the case of hydroxymethylation. Under acidic conditions formaldehyde will condense with uridine to give 5-hydroxymethyluridine (1; X = CH_2OH, R^1 = OH, R^2 = H) in moderate yield,[32] more forcing conditions being required for 2′-deoxyuridine. If 2′,3′-*O*-isopropylideneuridine (6; X = H, R = H) is used the reaction proceeds under basic conditions to give the isopropylidene derivative (6; X = CH_2OH, R = H) in high yield.[33,34] In this case, the conformation of the sugar may be such that the 5′-hydroxyl is more readily able to assist the reaction by attack at C(6), facilitating a Type 2 pathway. There are no published reports of the formation of 5-hydroxymethyluridine under alkaline conditions. Neither cytidine nor 2′,3′-*O*-isoptopylidene cytidine (7; X = H, R = H) gives the corresponding C(5)-hydroxymethyl derivatives when treated with formaldehyde. Instead, the N(4)-hydroxymethyl derivatives are formed reversibly.[35]

The hydroxymethyluridine nucleotide (1; X = CH_2OH, R^1 = OH, R^2 = H_2PO_3) may be prepared under acidic[36] or basic[37] catalysis, but reaction times are long (4—5 d) and yields are low (10—20%). 2′-Deoxycytidine 5′-phosphate will not condense with formaldehyde at C(5) in acid solution but 5-hydroxymethyldeoxycytidine 5′-phosphate (2; X = CH_2OH, R^1 = H, R^2 = H_2PO_3) is formed under base-catalysed conditions.[37] These reactions cannot proceed *via* a Type 2 mechanism involving the C(5′) hydroxyl, and so reaction proceeds less efficiently in the aqueous solvent by an intermolecular Type 2 pathway involving water.

[25] M. Grunberg-Manago and A. M. Michelson, *Biochim. Biophys. Acta*, 1964, **80**, 431.
[26] K. W. Brammer, *Biochim. Biophys. Acta*, 1963, **72**, 217.
[27] F. B. Howard, J. Frazier, and H. T. Miles, *J. Biol. Chem.*, 1969, **244**, 1291.
[28] M. A. W. Eaton and D. W. Hutchinson, *Biochemistry*, 1972, **11**, 3162.
[29] K. Kikugawa, I. Kawada, and M. Ichino, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 35.
[30] (*a*) P. K. Chang and A. D. Welch, *J. Medicin. Chem.*, 1963, **6**, 428; (*b*) A. Massaglia, U. Rosa, and S. Sosi, *J. Chromatog.*, 1965, **17**, 316.
[31] A. M. Michelson and C. Monny, *Biochim. Biophys. Acta*, 1967, **149**, 88.
[32] R. E. Cline, R. M. Fink, and K. Fink, *J. Amer. Chem. Soc.*, 1959, **81**, 2521.
[33] K. H. Scheit, *Chem. Ber.*, 1966, **99**, 3884.
[34] D. W. Hutchinson and T. K. Bradshaw, unpublished work.
[35] K. H. Scheit, *Tetrahedron Letters*, 1965, 1031.
[36] F. Maley, *Arch. Biochem. Biophys.*, 1962, **96**, 550.
[37] A. H. Alegria, *Biochim. Biophys. Acta*, 1967, **149**, 317.



(6)



(7)

Uridine and UMP react with formaldehyde and diethylamine to give the 5-diethylaminomethyl derivatives (1; X = CH_2NEt_2, R^1=OH, R^2 = H) and (1; X = CH_2NEt_2, R^1 = OH, R^2 = H_2PO_3) by a Mannich reaction;[38] this reaction most probably proceeds by a Type 1 mechanism.

D. Hydrogen Isotope Exchange.—Exchange of the hydrogen at C(5) of both uridine and cytidine derivatives has been observed to occur with either acid[39] or base[40,41] catalysis. In all cases, the reaction occurs by a Type 2 mechanism with initial attack at C(6) by a nucleophile (*e.g.* water or a sugar hydroxy-group). Base-catalysed exchange at C(5) can be accompanied by exchange of hydrogen at C(6) and this latter exchange has been explained as proceeding by a delocalized anion formed by direct abstraction of the C(6) proton.[41] Exchange of hydrogen at C(5) has also been observed during the photohydration of UMP.[42] This can occur by the addition of labelled water to the uridine nucleus by a Type 3 mechanism, followed by dehydration.

Uridine, cytidine, UMP, and CMP will add bisulphite across the 5,6-double bond[43] and this is the basis of another method of exchanging the C(5) hydrogen in these compounds by a Type 2 mechanism. With cytidine derivatives, however, the exchange reaction can be accompanied by extensive deamination.[44]

[38] E. I. Budovskii, V. N. Shibaev, and G. I. Eliseeva, in 'Synthetic Procedures in Nucleic Acid Chemistry', ed. W. W. Zorbach and R. S. Tipson, Wiley, New York, 1968, Vol. 1, p. 436.
[39] R. M. Fink, *Arch. Biochem. Biophys.*, 1964, **107**, 493; R. Shapiro and R. S. Klein, *Biochemistry*, 1967, **6**, 3576.
[40] W. J. Wechter and R. C. Kelly, *Coll. Czech. Chem. Comm.*, 1970, **35**, 1991.
[41] W. J. Wechter, *Coll. Czech. Chem. Comm.*, 1970, **35**, 2003; J. A. Rabi and J. J. Fox, *J. Amer. Chem. Soc.*, 1973, **95**, 1628.
[42] R. W. Chambers, *J. Amer. Chem. Soc.*, 1968, **90**, 2192.
[43] K. Kai, Y. Wataya, and H. Hayatsu, *J. Amer. Chem. Soc.*, 1971, **93**, 2089.
[44] Y. Wataya and H. Hayatsu, *Biochemistry*, 1972, **11**, 3583; M. Sono, Y. Wataya, and H. Hayatsu, *J. Amer. Chem. Soc.*, 1973, **95**, 4745.

E. Nitration and Thiolation.—Nitration of a sugar-protected uridine with a mixture of concentrated nitric and sulphuric acids gives the 5-nitro-derivative, which after deprotection yields 5-nitrouridine (1; X = NO_2, R^1 = OH, R^2 = H).[45] Under these conditions, the reaction most probably proceeds by a Type 1 mechanism and it is interesting to note that attempts to apply this procedure to the synthesis of 5-nitrocytidine were unsuccessful.[46] The nucleotide, 5-nitro-UMP (1; X = NO_2, R^1 = OH, R^2 = H_2PO_3), is readily prepared from fully protected UMP and nitronium tetrafluoroborate[47] by a Type 1 mechanism. This reaction does not proceed with CMP.

Uridine and 2′-deoxyuridine will react with thiocyanogen chloride to give the 5-thiocyanato-nucleosides (1; X = SCN, R^1 = OH, R^2 = H) and (1; X = SCN, R^1 = R^2 = H).[48] Electrophilic attack by thiocyanogen chloride, a pseudo-halogen, followed by elimination will give the product by a Type 1 reaction. The evidence available is not sufficient to rule out a Type 2 mechanism. Once again, attempts to extend this reaction to cytidine and N^4-acetylcytidine were unsuccessful.[49]

F. Mercuration.—The nucleosides and nucleotides of uracil and cytosine react readily with mercuric acetate at 55 °C to give products in which, as ^{1}H n.m.r., elemental, electrophoretic, and chromatographic analyses have shown, the mercury atom is covalently bound at C(5), (1; X = $HgMeCO_2$, R^1 = H or OH, R^2 = H or H_2PO_3).[50a] The products probably arise by a Type 1 mechanism and this reaction has been carried out at the polynucleotide level.[50b]

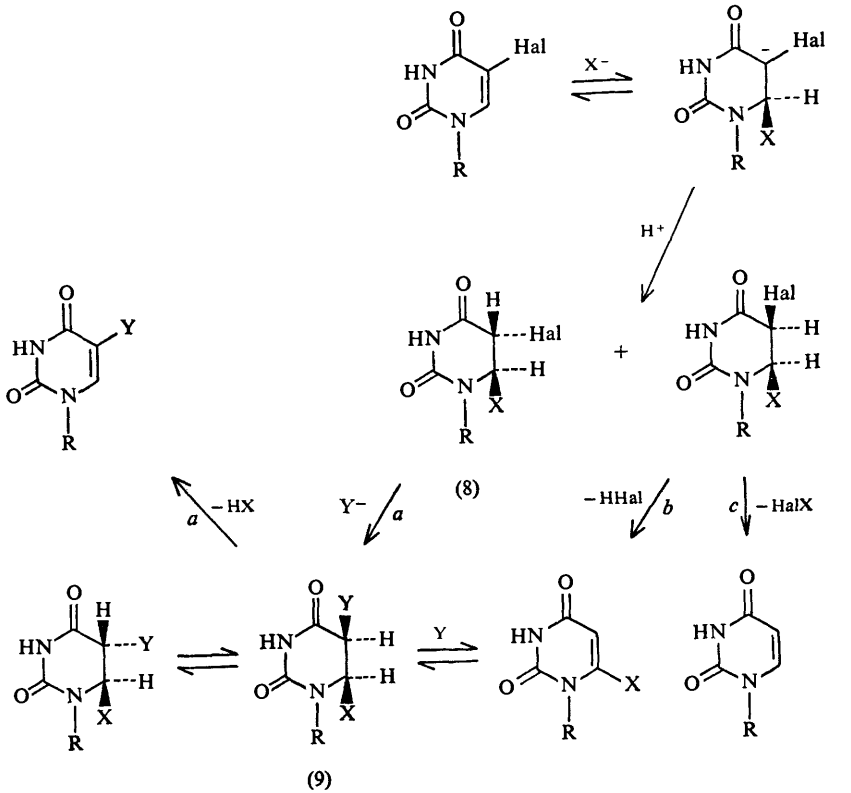
3 Displacement of Halogen at C(5)

A. Reaction Mechanism.—The introduction of a halogen substituent at C(5) of pyrimidine nucleosides and nucleotides offers a means of further functionalization at this position by nucleophilic displacement. Nucleophilic displacement in 5-halogenopyrimidine nucleosides or nucleotides can lead to a complex mixture of products and, as with halogenation, the nature of the products isolated makes speculation on the reaction mechanisms difficult. More than one pathway can be envisaged for this displacement reaction but it is unlikely that direct substitution of halogen occurs from the pyrimidine. No evidence has been published so far for the involvement of an aryne in this reaction and the

[45] I. Wempen, I. L. Doerr, L. Kaplan, and J. J. Fox, *J. Amer. Chem. Soc.*, 1960, **82**, 1624.
[46] J. J. Fox and D. Van Praag, *J. Org. Chem.*, 1961, **26**, 526.
[47] V. K. Shibaev, G. I. Eliseeva, and N. K. Kochetkov, *Doklady Akad. Nauk S.S.S.R.*, 1972, **203**, 860 (*Chem. Abs.*, 1972, **77**, 34 819).
[48] T. Nagamachi, P. F. Torrence, J. A. Waters, and B. Witkop, *J.C.S. Chem. Comm.*, 1972, 1025.
[49] T. Nagamachi, J. L. Fourrey, P. F. Torrence, J. A. Waters, and B. Witkop, *J. Medicin. Chem.*, 1974, **17**, 403.
[50] (*a*) R. M. K. Dale, D. C. Livingston, and D. C. Ward, *Proc. Nat. Acad. Sci. U.S.A.*, 1973, **70**, 2238; (*b*) R. M. K. Dale, E. Martin, D. C. Livingston, and D. C. Ward, *Biochemistry*, 1975, **14**, 2447.

most likely pathways involve nucleophilic addition at C(6) followed by displacement of halide.

5-Halogenopyrimidine nucleosides and nucleotides can react with nucleophiles to give exclusively the 5- or 6-substituted products, a mixture of the two, or the dehalogenated derivative. These products can all be accounted for if the reaction is assumed to follow one of the pathways illustrated in Scheme 4.



Scheme 4

The first step in the formation of all the products is probably nucleophilic attack by XH or X^- at C(6) to give the intermediate (8) possibly as a pair of epimers. Direct displacement of halide from one epimer leads to (9) and elimination of HX from (9) leads to a 5-substituted pyrimidine (pathway *a*). Alternatively loss of HHal from the other epimer gives a 6-substituted pyrimidine (pathway *b*). If the nucleophile X has electron-withdrawing properties (*e.g.* X = CN), further reaction can occur at C(5), leading to (9). Loss of HX as above then occurs leading to the 5-substituted pyrimidine.

A third pathway (*c*) can also be followed when XHal is eliminated from (8). In this case the product is the dehalogenated pyrimidine derivative.

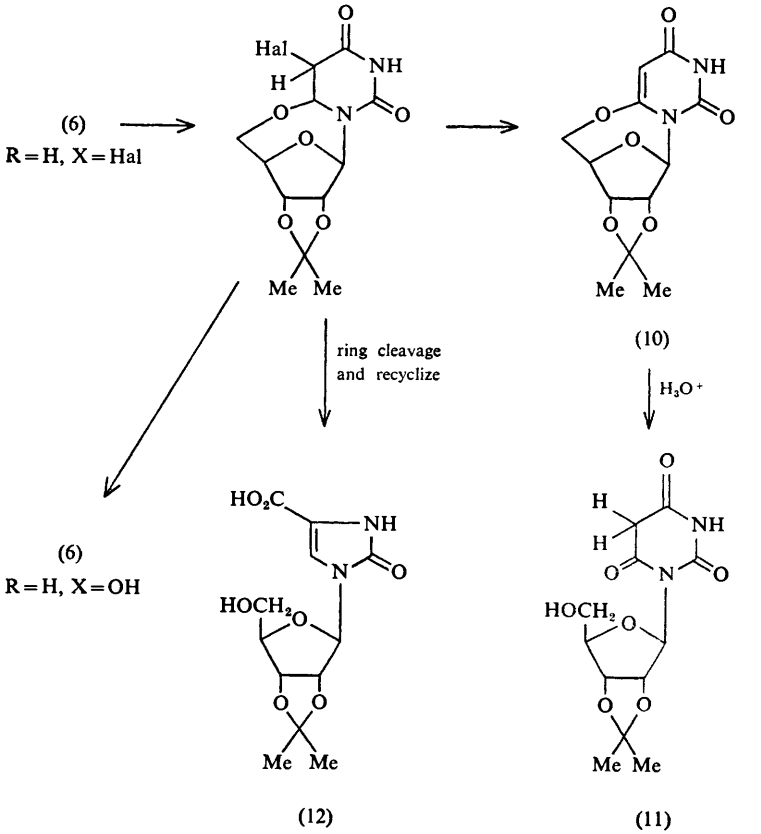
B. Displacement Reactions.—The formation of 5-hydroxy-UMP and -UDP from the reaction between UMP or UDP and aqueous bromine in the presence of a base[16] is an example of pathway *a* mentioned in the previous section. The additions of methyl hypobromite to 1-methyluracil,[51] 5-fluorouridine,[52] or thymidine[3] are other examples of this addition reaction. In these cases the intermediates can be purified and intermediates such as (4; R^1 = H, R^2 = H_2PO_3, R^3 = Me) can be isolated from the reaction between methyl hypobromite and dUMP. Treatment of (4; R^1 = H, R^2 = H_2PO_3, R^3 = Me) with sodium disulphide followed by reduction gives 5-mercapto-dUMP (1; X = SH, R^1 = H, R^2 = H_2PO_3),[3] which can be explained by the reaction following pathway *a*. However, attempts at nucleophilic displacement by fluoride,[20] methoxide,[47] or azide[34] of the C(5) bromine from the methyl hypobromite adducts of uracil, uridine, or UMP have been unsuccessful. Little work has been reported on 5,6-dihydro-addtion products of methyl hypobromite and cytidines. Polycytidylic acid, however, does add methyl hypobromite and the intermediate reacts with sodium disulphide to give poly-(5-mercaptocytidylic acid).[53] Treatment of cytidine with aqueous bromine followed by chromatography on a basic ion-exchange resin leads to 5-hydroxycytidine and this reaction may follow pathway *a*.[54] Treatment of cytidine nucleotides with aqueous bromine in the presence of a tertiary base also results in the formation of the 5-hydroxycytidine derivative provided reaction times are kept short to avoid deamination of the cytidine.[55,56]

As is the case in the Type 2 substitution pathway, the nucleophile which adds on to C(6) of a nucleoside during a displacement reaction can be the 5′-hydroxyl of the sugar itself. O^6-5′-Cyclonucleosides, *e.g.* (10), have been suggested as intermediates in the base-catalysed exchange of H(5) in uridine nucleosides.[57,58] It is interesting to note that little or no exchange occurs of H(5) in 1-methyluracil or with 5′-deoxynucleosides,[57] which provides confirmatory evidence for the participation of the 5′-hydroxy-group in this reaction. The base-catalysed exchange reaction in 2′,3′-*O*-isopropylideneuridine is appreciably faster than with uridine itself and presumably the conformation of the sugar must be altered so as to facilitate attack at C(6) by the 5′-hydroxy-group.[58] O^6-5′-Cyclonucleosides have been implicated in a number of other displacement reactions of 5-halogenopyrimidine nucleosides[59] and these cyclonucleosides have been

[51] L. Szabo, T. I. Kalman, and J. T. Bardos, *J. Org. Chem.*, 1970, **35**, 1434.
[52] R. Duschinsky, T. Gabriel, W. Tautz, A. Nussbaum, M. Hoffer, E. Grunberg, J. H. Burchenal, and J. J. Fox, *J. Medicin. Chem.*, 1967, **10**, 47.
[53] P. Chandra, U. Ebener, and A. Götz, *F.E.B.S. Letters*, 1975, **53**, 10.
[54] T. K. Fukuhara and D. W. Visser, *Biochemistry*, 1962, **1**, 563.
[55] G. E. Means and H. Fraenkel-Conrat, *Biochim. Biophys. Acta*, 1971, **247**, 441.
[56] M. A. W. Eaton and D. W. Hutchinson, *Biochim. Biophys. Acta*, 1973, **319**, 281.
[57] D. V. Santi and C. F. Brewer, *J. Amer. Chem. Soc.*, 1968, **90**, 6326.
[58] R. J. Cushley, S. R. Lipsky, and J. J. Fox, *Tetrahedron Letters*, 1968, 5393.
[59] P. K. Chang, *J. Org. Chem.*, 1965, **30**, 3913.

isolated in certain instances,[60,61] notably in the reaction between 5-halogenopyrimidine nucleosides and cyanide ion[62] which will be discussed in more detail below.

5-Halogenouridine nucleosides are degraded rapidly in aqueous alkaline media.[29,61] In addition to displacement and O^6-5′-cyclonucleoside formation mentioned above, further reactions can occur (Scheme 5). Hydrolysis of the cyclonucleoside (10) gives a nucleoside of isobarbituric acid (11), and cleavage of the pyrimidine ring can take place leading to ring contraction and the formation of (12), which could also arise by a pathway related to the Favorskii reaction.[61] 5-Halogenocytidine nucleosides are deaminated more readily in aqueous



Scheme 5

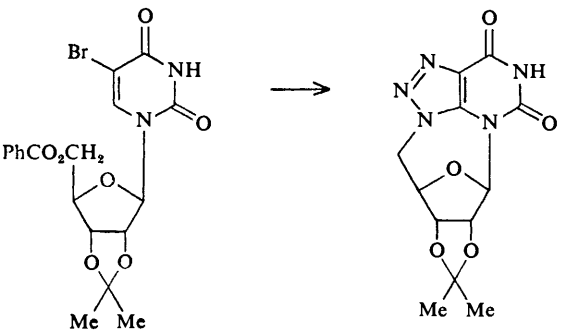
[60] D. Lipkin, C. Cori, and M. Sano, *Tetrahedron Letters*, 1968, 5993.
[61] B. A. Otter, E. A. Falco, and J. J. Fox, *J. Org. Chem.*, 1969, **34**, 1390.
[62] T. Ueda, H. Inoue, and A. Matsuda, *Ann. New York Acad. Sci.*, 1975, **255**, 121.

alkali than the parent unsubstituted compounds, and the 5-halogenouridines once formed can participate in the reactions outlined above.[29]

While aqueous ammonia can give rise to a complex mixture of products with 5-bromouridines, anhydrous liquid ammonia reacts to give the 5-aminouridine nucleosides as the only isolable products.[6,63,64] Other amines, *e.g.* morpholine[16a] or dimethylamine,[65] will react with 5-bromouridines and anhydrous dimethylamine will displace bromide from 5-bromo-CMP.[66] The most reasonable pathway for this reaction is path *a* (Scheme 4).

The reaction between 5-halogenopyrimidine nucleosides and cyanide ion in DMF follows pathway *b* as both 5- and 6-substituted products are formed.[62,67,68] O^6-5′-Cyclonucleosides are also formed, probably owing to attack by the 5′-hydroxyl on C(6) of the 6-cyanonucleoside followed by elimination of HCN.[62] 5-Iodouracil reacts with cuprous cyanide in DMF on heating to give uracil, presumably by pathway *c*. When the NH and OH protons of 5-iodouracil or 5-iodo-2′-deoxyuridine are protected by silylation, displacement of the iodine by cyanide ion occurs and 5-cyanouracil or 5-cyano-2′-deoxyuridine is formed.[68]

When azide ion is used in place of cyanide in the reaction with 2′,3′-*O*-isopropylidene-5-bromouridine, no 5-azidouridine derivatives can be detected in the intractable mixture of products.[34] Treatment of 5′-*O*-benzoyl-2′,3′-*O*-isopropylidene-5-bromouridine with azide ion in DMF gave 5′-substitution followed by intramolecular attack at C(6) of the uridine and displacement of halide ion to give (13); a similar product is formed from 2′,3′-*O*-isopropylidene-



(13)

[63] M. Roberts and D. W. Visser, *J. Amer. Chem. Soc.*, 1952, **74**, 668.
[64] (*a*) R. Lührmann, U. Schwarz, and H. G. Gassen, *F.E.B.S. Letters*, 1973, **32**, 55; (*b*) W. Hillen and H. G. Gassen, *Biochim. Biophys. Acta*, 1975, **407**, 347.
[65] T. Ueda, *Chem. and Pharm. Bull. (Japan)*, 1962, **10**, 788.
[66] J. O. Folayan and D. W. Hutchinson, *Tetrahedron Letters*, 1973, 5077.
[67] (*a*) H. Inoue and T. Ueda, *Chem. and Pharm. Bull. (Japan)*, 1971, **19**, 1743; (*b*) S. Senda, K. Hirota, and T. Asao, *J. Org. Chem.*, 1975, **40**, 353.
[68] R. C. Bleackley, A. S. Jones, and R. T. Walker, *Nucleic Acids Res.*, 1975, **2**, 683.

5′-*O*-mesyl-5-bromocytidine.[69] However, no reaction occurs with 2′,3′-*O*-isopropylidene-5′-*O*-trityl-5-bromocytidine, which supports the suggestion that attack by azide must occur initially at C(5′) rather than C(6).

The reaction of 5-halogenopyrimidine nucleosides and nucleotides with sulphur nucleophiles can follow any of the pathways outlined in Scheme 4. Sodium disulphide will not displace bromide from 5-bromouridine although the 5-mercapto-derivative is produced from 5′-*O*-acetyl-2′,3′-*O*-isopropylidene-5-bromouridine.[70] Cysteine will react with 2′-deoxy-5-bromouridine to give a mixture of the 5-substituted and the dehalogenated nucleosides.[71] Pathway *b* is presumably followed in the reaction of 5-halogenocytidine nucleosides and ethylmercaptan in the presence of cyanide ion when the 5-ethylmercapto-derivative is the major product.[62]

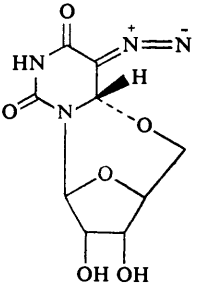
Dehalogenation by pathway *c* is the major route in the reaction of 5-bromo-nucleosides with bisulphite.[72] This reaction has been extensively studied for 5-bromouracil,[73] 5-bromouridine,[74] 5-bromodeoxyuridine,[75] and 5-halogeno-cytosines.[76] In all cases, it appears that debromination proceeds *via* a 5,6-dihydro-5-bromo-6-sulphonate intermediate, although this has not been shown for 5-bromouridine. Kinetic studies reveal that the mechanism of debromination is obviously different for pyrimidine bases and nucleosides,[75] and 5-bromouracil reacts more rapidly than the nucleoside, suggesting that the 5′-hydroxyl of the nucleoside might compete with bisulphite in the attack at C(6).

Diazotization of 5-aminouridine gives 5-diazouridine[63] by analogy to the reaction of 5-aminouracil.[77] Several different structures have been proposed for these compounds based on different information.[78] Currently accepted structures for 5-diazouridine (14) and 5-diazouracil (15) were suggested by Thurber and Townsend on the basis of ^{1}H n.m.r. data.[79]

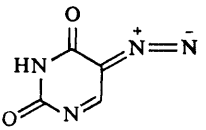
Nucleophilic displacements of the diazo-group in (15) have been reported. For example, treatment with thiourea gives 5-thiouracil.[80] 5-Iodouracil has been prepared from elemental iodine and (15)[81] while cuprous halides react with (15) to give 5-halogenouracils.[82] This can be considered as an analogue

[69] T. Sasaki, K. Minamota, M. Kino, and T. Mizuno, *J. Org. Chem.*, 1976, **41**, 1100.
[70] H. Inoue, S. Tomita, and T. Ueda, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 2614.
[71] Y. Wataya, K. Negishi, and H. Hayatsu, *Biochemistry*, 1973, **12**, 3992.
[72] H. Hayatsu, *Progr. Nucleic Acid Res.*, 1976, **16**, 75.
[73] (*a*) G. S. Rork and I. H. Pitman, *J. Amer. Chem. Soc.*, 1975, **97**, 5566; (*b*) R. Shapiro, M. Welcher, V. Nelson, and V. Di Fate, *Biochim. Biophys. Acta*, 1976, **425**, 115; (*c*) F. A. Sedor, D. G. Jacobson, and E. G. Sander, *J. Amer. Chem. Soc.*, 1975, **97**, 5572.
[74] J. Fourrey, *Bull. Soc. chim. France*, 1972, 4580.
[75] H. Hayatsu, T. Chikuma, and K. Negishi, *J. Org. Chem.*, 1975, **40**, 3862.
[76] D. G. Jacobson, F. A. Sedor, and E. G. Sander, *Bio-org. Chem.*, 1975, **4**, 72.
[77] T. B. Johnson, O. Baudisch, and A. Hoffmann, *Chem. Ber.*, 1931, **64**, 2629.
[78] (*a*) F. G. Fisher and E. Fahr, *Annalen*, 1962, **651**, 64; (*b*) J. P. Paolini, R. K. Robins, and C. C. Cheng, *Biochim. Biophys. Acta*, 1963, **72**, 114.
[79] T. C. Thurber and L. B. Townsend, *J. Heterocyclic Chem.*, 1972, **9**, 629.
[80] T. J. Bardos, R. R. Herr, and T. Enkoji, *J. Amer. Chem. Soc.*, 1955, **77**, 960.
[81] J. Gut, J. Morávek, C. Párkányi, M. Prystas, J. Škoda, and F. Šorm, *Coll. Czech. Chem. Comm.*, 1959, **24**, 3154.
[82] S. H. Chang, I. K. Kim, D. S. Park, and B. S. Hahn, *Daehan. Hwahak Hwoejee*, 1965, **9**, 29 (*Chem. Abs.*, 1966, **64**, 15 876).

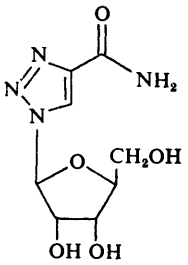
of the Sandmeyer reaction. According to one report[83] potassium cyanide and (15) give rise to 5-cyanouracil, but later workers were unable to substantiate this result.[69]



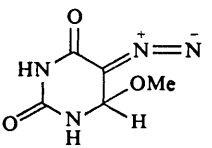
(14)



(15)



(16)



(17)

Attempts at nucleophilic displacements in O^6-5′-cyclo-5-diazouridine (14) indicated that they did not occur at low temperatures. At higher temperatures, in aqueous acetonitrile, ring contraction occurs to afford the triazole (16).[84] The deoxyuridine derivative and 5-diazouracil-6-methanolate (17) react analogously and it appears that reaction proceeds *via* initial attack of water at C(2) followed by cleavage of the N(1)—C(2) bond. Treatment of 5-diazouridine with dimethylamine as nucleophile results in coupling and the formation of 5-(3,3-dimethyl-1-triazeno)uridine (1; X = $C_2H_6N_3$, R^1 = OH, R^2 = H).[79]

4 Functionalization at C(5) and Other Reactions

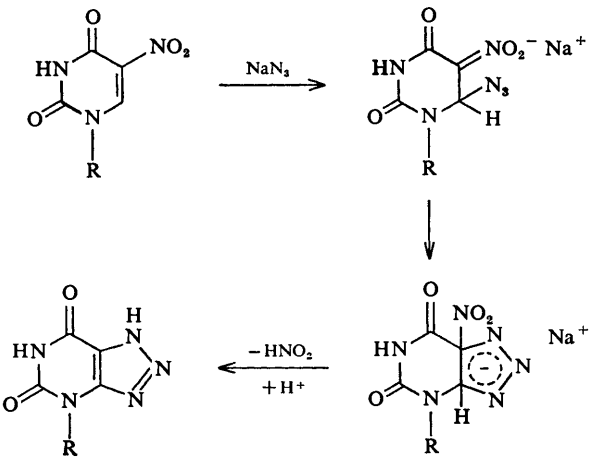
5-Hydroxymethyluridine (1; X = CH_2OH, R^1 = OH, R^2 = H) can be function-

[83] S. H. Chang, J. S. Kim, and T. S. Huh, *Daehan. Hwahak Hwoejee*, 1969, **13**, 177 (*Chem. Abs.*, 1969, **71**, 112 880).

[84] T. C. Thurber and L. B. Townsend, *J. Org. Chem.*, 1976, **41**, 1041.

alized *via* the methyl bromide,[85] oxidized to the aldehyde,[86] or reduced to thymidine.[38] Recently a method was described for introducing homologous alkyl substituents at C(5) of nucleosides.[87] Treatment of 5-chloromercuriuridine (1; X = HgCl, R^1 = OH, R^2 = H) with $LiPdCl_2$ and ethylene gave an intermediate which was reduced by sodium borohydride to 5-ethyluridine (1; X = Et, R^1 = OH, R^2 = H). With allyl chloride as substrate, 5-allyluridine (1; X = $CH_2CH{=}CH_2$, R^1 = OH, R^2 = H) is formed. It is possible that the 5-mercurinucleosides and nucleotides may prove useful intermediates for the synthesis of 5-substituted derivatives. The mercurinucleotides, for example, are readily converted into the 5-halogenonucleotides.[88]

The reaction between 5-nitrouridine or 5-nitrocytidine and sodium azide leads to the formation of 3-β-D-ribofuranosyl-8-azaxanthine and -8-azaisoguanosine respectively (Scheme 6).[89] Nitrous acid is liberated in this reaction which must proceed by initial attack by azide ion at C(6) followed by cyclization and loss of nitrous acid.



Scheme 6

5 Biochemical Examples

A. Thymidylate Synthetase.—An important step in the biosynthesis of DNA is the conversion of dUMP into dTMP prior to the incorporation of thymine

[85] J. Farkaš and F. Šorm, *Coll. Czech. Chem. Comm.*, 1969, **34**, 1696.
[86] (*a*) V. W. Armstrong and F. Eckstein, *Nucleic Acids Res. Special Pub.*, 1975, **1**, 597; (*b*) V. W. Armstrong, J. K. Dattagupta, F. Eckstein, and W. Saenger, *Nucleic Acids Res.*, 1976, **3**, 1791.
[87] D. E. Bergstrom and J. L. Ruth, *J. Amer. Chem. Soc.*, 1976, **98**, 1587.
[88] R. M. K. Dale, D. C. Ward, D. C. Livingston, and E. Martin, *Nucleic Acids Res.*, 1975, **2**, 915.
[89] H. U. Blank and J. J. Fox, *J. Amer. Chem. Soc.*, 1968, **90**, 7175.

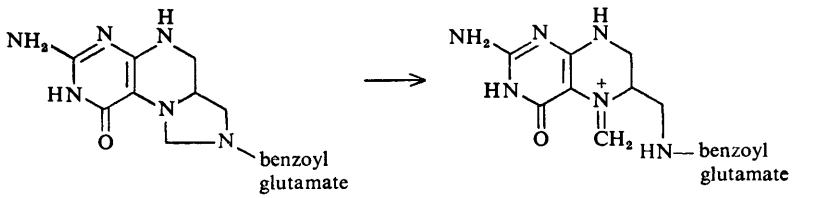
into the DNA. This reaction, which results in the replacement of the hydrogen atom at C(5) in dUMP by a methyl group, is catalysed by the enzyme thymidylate synthetase which occurs in both bacteria and animals. The mechanism of action of this enzyme has been the subject of many investigations and a reasonable mechanistic scheme has been put forward for this enzymic reaction which incorporates some of the suggested reaction pathways mentioned earlier in this review.

Tetrahydrofolate is an essential cofactor for thymidylate synthetase and is dehydrogenated to 7,8-dihydrofolate. Formaldehyde is the source of the methyl group in dTMP and the following overall reaction can be written:[90]

tetrahydrofolate + CH_2O ⇌ N^5N^{10}-methylenetetrahydrofolate + H_2O

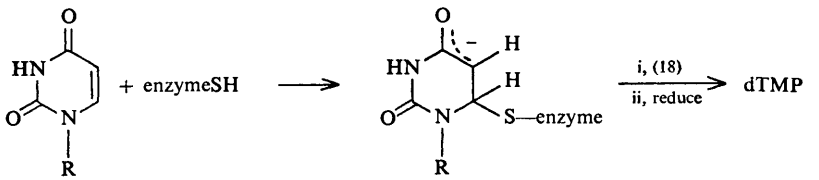
N^5N^{10}-methylenetetrahydrofolate + dUMP ⇌ dTMP + dihydrofolate

The methylene group in N^5N^{10}-methylenetetrahydrofolate (18) itself may not be sufficiently electrophilic for attack to occur at C(5) in dUMP and it may be that the cationic imine (19)[91] is the reactive species. The biosynthesis of dTMP



(18) (19)

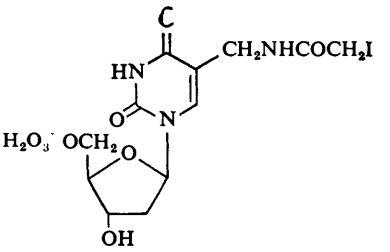
can then be regarded as an example of a Type 2 substitution reaction at C(5) of dUMP followed by a reductive step (Scheme 7). Thymidylate synthetase is stimulated by exogenous thiols and activity is lost if the enzyme is treated with SH reagents such as *p*-chloromercuribenzoate.[92] This has led to the suggestion that the SH group of a cysteinyl residue in thymidylate synthetase adds to C(6) of dUMP, assisting the attack on the exocyclic methylene group in (19).[90]



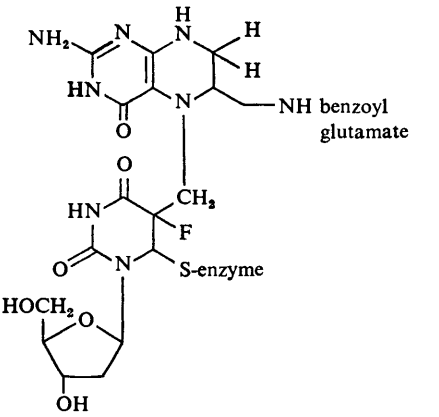
Scheme 7

[90] M. Friedkin, *Adv. Enzymol.*, 1973, **38**, 235.
[91] R. G. Kallen and W. P. Jencks, *J. Biol. Chem.*, 1966, **241**, 5851.
[92] R. B. Dunlap, N. G. L. Harding, and F. M. Huennekens, *Biochemistry*, 1971, **10**, 88.

5-Iodacetamidomethyl-2′-deoxyuridine 5′-phosphate (20) will irreversibly inhibit thymidylate synthetase from Ehrlich ascites tumours but not the enzyme from calf thymus.[93] The two thymidylate synthetases differ in molecular weight and it has been suggested that structural differences may exist between the two forms of the enzyme so that although (20) binds to the thymus enzyme it is unable to alkylate the reactive portion of this enzyme (*e.g.* a thiol group).



(20)



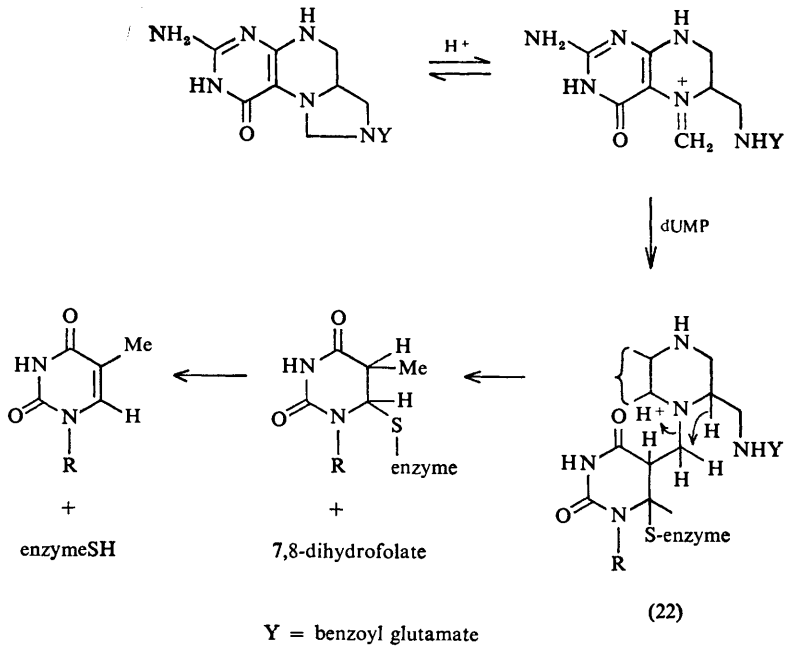
(21)

5-Fluoro-dUMP is a powerful inhibitor of thymidylate synthetase and a covalent complex (21) is formed between (18), 5-fluoro-dUMP, and the enzyme in which the uridine nucleotide is joined to the active site of the enzyme through a cysteinyl residue attached to C(6) of the pyrimidine ring.[94] As has been mentioned earlier, sulphur nucleophiles can cause debromination of 5-bromouracil and its nucleosides following attack at C(6) of the pyrimidine ring.[71,95] Thymidylate synthetase will also catalyse the debromination of 5-bromo-dUMP[96] presumably by a similar mechanism involving a cysteinyl residue of the enzyme. It is interesting to note that neither bisulphite[97] nor thymidylate synthetase[96] will displace fluoride from 5-fluoro-dUMP; this is an important factor in the biological activity of 5-fluoro-dUMP as will be discussed below.

A reduction step is involved in the formation of the methyl group in dTMP and tracer studies indicate that the third hydrogen of the methyl group comes

[93] R. L. Barfknecht, R. A. Huet-Rose, A. Kampf, and M. P. Mertes, *J. Amer. Chem. Soc.*, 1976, **98**, 5041.
[94] P. V. Danenburg, R. J. Langenbach, and C. Heidelberger, *Biochemistry*, 1974, **13**, 926; D. V. Santi, C. S. McHenry, and H. Sommer, *Biochemistry*, 1974, **13**, 471; A. L. Pogolotti, jun., K. M. Ivanetich, H. Sommer, and D. V. Santi, *Biochem. Biophys. Res. Comm.*, 1976, **70**, 972.
[95] F. A. Sedor and E. G. Sander, *Arch. Biochem. Biophys.*, 1974, **161**, 632.
[96] Y. Wataya and D. V. Santi, *Biochem. Biophys Res. Comm.*, 1975, **67**, 818.
[97] E. G. Sander and C. L. Deyrup, *Arch. Biochem. Biophys.*, 1972, **150**, 600.

from C(6) of the tetrahydrofolate and not from an external cofactor.[91] Transfer of tritium from [C(6)-^{3}H]-(18) to dTMP occurs intramolecularly in the intermediate[75,98] and a kinetic isotope effect has been observed indicating that hydrogen transfer is the rate-determining step in the enzymic reaction. The hydrogen at C(5) in dUMP is lost to water[99] and the complete reaction sequence for thymidylate synthetase can be written as shown in Scheme 8.



Scheme 8

The proposed mechanism explains the effectiveness of 5-fluorouracil and 5-fluoro-2′-deoxyuridine as anticancer agents.[100] Both compounds are converted *in vivo* into 5-fluoro-dUMP which forms a ternary complex similar to (21) with thymidylate synthetase in cancer cells. Since the C—F bond cannot be broken the synthesis of thymidine cannot occur and DNA synthesis is blocked. Whereas 5-fluoro-2′-deoxyuridine is ineffective as an antiviral agent *in vivo*, 5-bromo- and 5-iodo-2′-deoxyuridine are antiviral agents and have been used against *Herpes simplex* virus.[101] The reason for this difference in antiviral activity is

[98] R. L. Blakley, B. V. Ramasastri, and B. M. McDougall, *J. Biol. Chem.*, 1963, **238**, 3075.
[99] M. I. S. Lomax and R. G. Greenberg, *J. Biol. Chem.*, 1967, **242**, 109.
[100] T. Kalman, *Ann. New York Acad. Sci.*, 1975, **255**, 326.
[101] J. Sugar and H. E. Kaufman, in 'Selective Inhibitors of Viral Functions', ed. W. A. Carter, C. R. C. Press, Cleveland, Ohio, 1973, p. 295.

not clear. Apparently the block in thymidylate synthetase activity caused by 5-fluorodeoxyuridine can be by-passed using thymidine obtained from salvage or breakdown mechanisms, and viral DNA synthesis occurs, but 5-fluorodeoxyuridine is not incorporated. On the other hand 5-bromo- and 5-iododeoxyuridines are incorporated into viral DNA and appear to cause a reduction in the number of infectious viral particles.[102] Incorporation of 5-bromo- and 5-iodouridine into DNA increases its lability to u.v. radiation and such an effect in a virus without a DNA repair mechanism would be lethal.[103]

5-Hydroxymethylcytosine occurs in the DNA of T-even bacteriophages which infect *Escherichia coli*, and deoxycytidylate hydroxymethyltransferase, the enzyme which catalyses the synthesis of this pyrimidine, has been isolated and characterized.[104] As in the case of thymidylate synthetase, N^5N^{10}-methylenetetrahydrofolate is a cofactor for the enzyme together with dCMP. The overall reaction can be written:

$$\text{dCMP} + (18) + H_2O \rightleftharpoons \text{5-hydroxymethyl dCMP} + \text{tetrahydrofolate}$$

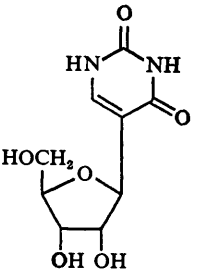
No detailed studies on the enzyme mechanism have been made but it is tempting to speculate that a covalent intermediate similar to (22) is formed between the enzyme, (19), and dCMP and that attack by water occurs at the bridge methylene group rather than intramolecular hydride transfer. This would explain the production of tetrahydro- rather than dihydro-folate as an end product of the reaction. A similar enzyme catalysing the formation of 5-hydroxymethyl-dUMP from dUMP has also been detected in certain bacteriophages.[105]

B. Methylation.—Ribosylthymine and 5-methylcytosine are minor components of tRNA and DNA. Some details of their biosynthesis have been established[106] and the methylation occurs at the macromolecular rather than the mononucleotide level. The conformation of the nucleic acid appears to play an important part in determining which base is methylated enzymically. *S*-Adenosylmethionine rather than a folate derivative is the donor of the methyl groups for these bases, and the reaction is a biochemical analogue of Type 1 electrophilic substitution.

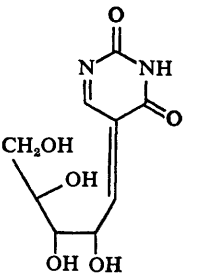
C. Pseudouridine.—Another minor constituent of tRNA is pseudouridine (23), an isomer of uridine in which the ribose is joined by a glycosidic bond to C(5) rather than N(1) of the uracil. The chemistry[107] and biochemistry[108] of pseudouridine have been well reviewed up to 1966 and space does not permit a detailed account of the preparation and properties of this nucleoside to be given here. As a consequence of the blocking of C(5) pseudouridine reacts with electrophiles

[102] A. S. Kaplan and T. Ben-Porat, *J. Mol. Biol.*, 1966, **19**, 320.
[103] W. D. Rupp and W. H. Prusoff, *Nature*, 1964, **202**, 1288.
[104] C. K. Mathews, F. Brown, and S. S. Cohen, *J. Biol. Chem.*, 1964, **239**, 2957.
[105] D. H. Roscoe and R. G. Tucker, *Biochem. Biophys. Res. Comm.*, 1964, **16**, 106.
[106] S. K. Keur and E. Borek, in 'The Enzymes' ed. P. D. Boyer, 3rd Edn., Academic Press, New York, 1974, Vol. 9, p. 167.
[107] R. W. Chambers, *Progr. Nucleic Acid Res.*, 1966, **5**, 349.
[108] E. Goldwasser and R. L. Heinrikson, *Progr. Nucleic Acid Res.*, 1966, **5**, 399.

preferentially at N(1). Thus acrylonitrile reacts rapidly to give 1-cyanoethyl-pseudouridine, which then reacts slowly with more acrylonitrile to give the 1,3-biscyanoethyl derivative.[107] A further feature of the chemistry of pseudouridine is the ready isomerization in acid or alkali of the naturally occurring β-ribofuranosyl nucleoside to the α-ribofuranosyl together with the α- and β-ribopyranosyl nucleosides. This does not occur to any appreciable extent with uridine and is presumably a measure of the stability of the intermediate (24).



(23)



(24)

Pseudouridine is formed in tRNA by the rearrangement of uridine residues in precursor tRNA. Biosynthetic studies[109] with cultures of *Streptoverticillicum ludakanus* indicate that pseudouridine arises by an intramolecular rearrangement of uridine and not by the formation of a 1,5-diribosyl intermediate. The presence of pseudouridine in tRNA has profound consequences on the conformation of the polynucleotide chain and may play a role in recognition of tRNA as it is absent from yeast initiator $tRNA_f$.[110]

The authors wish to thank the Medical Research Council for financial support.

[109] T. Uematsu and R. J. Suhadolnik, *Biochim. Biophys. Acta*, 1973, **319**, 348.
[110] M. Simsek and U. L. Raj Bhandary, *Biochem. Biophys. Res. Comm.*, 1972, **49**, 508.