PYRIMIDINE NUCLEOSIDE TRANSFORMATIONS VIA ANHYDRONUCLEOSIDES

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The field of nucleosides has undergone rapid growth and development during the past decade[1-3]. Whereas, earlier studies were concerned with the development of methods for the synthesis of the nucleoside components of the nucleic acids, today several methods are available—each of which has its advantages and limitations. The host of "fraudulent" or "anomalous" nucleosides available today attests to the ability of chemists to cope with this interesting and rather complex group of compounds. Nor have their efforts been unrewarding. It is obvious to all that nucleosides have provided their fair share of interesting biochemicals useful in the elucidation of biosynthetic pathways and helpful both as anti-neoplastic and anti-viral agents. In addition, the increasing amount of "odd" nucleosides found as minor components of certain nucleic acids[4], especially of transfer RNA, has further stimulated chemical efforts in this area. Finally, the continuing discovery of nucleoside antibiotics[5] (of which there are now over twenty) has served to introduce an increasing number of natural-products chemists into this burgeoning field.

This discussion deals with but one aspect of our research during the past few years in the area of the pyrimidine nucleosides and is not intended as a comprehensive review. Specifically, it covers that aspect of our endeavours involved in the use of *preformed* nucleosides, natural or anomalous, as precursors for the synthesis of new nucleosides. As with most workers in this area, our studies are motivated by biochemical or biological interests, and reference to such considerations will be made in this discussion.

Nucleoside chemistry is replete with examples from many laboratories of nucleoside transformations which involved alteration only of the aglycon portion, that is, reactions in which the sugar moiety played a "passive" role. Halogenation, methylation, thiation, deamination, etc. of the main nucleosides of the nucleic acids (*Figure 1*) and of others are well documented[1-3]. Our main interest however was in alterations in the carbohydrate moieties of nucleosides. One of the significant developments in nucleoside chemistry was the earlier discoveries[6] by Brown, Michelson, Todd and their associates at Cambridge who showed that pyrimidine anhydronucleosides (or "cyclonucleosides") could be formed which involve an oxygen bridge between carbon-2 of the aglycon and the sugar moiety. We will discuss the chemistry of these and other types of anhydronucleosides as tools for stereochemically-controlled reactions involving:

(a) alteration of the configuration of the sugar moiety;

(b) introduction of functional groups into the sugar moiety;

(c) alterations of the aglycon of nucleosides including rearrangement of the heterocyclic ring.

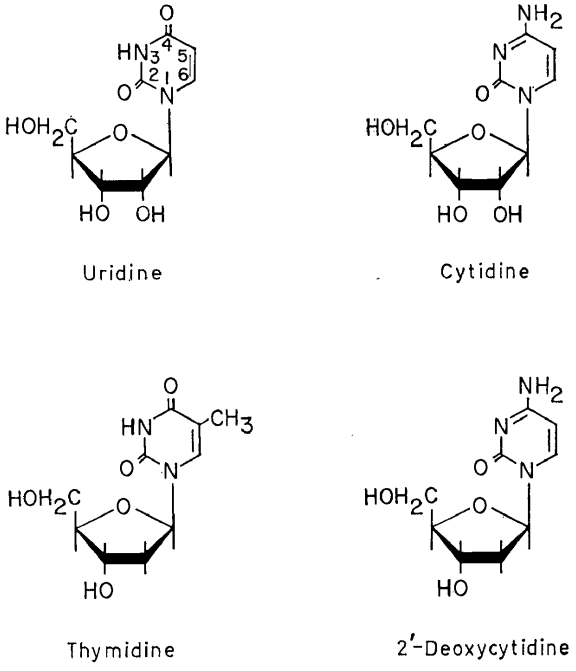


Figure 1. The main pyrimidine nucleosides in the nucleic acids.

It became clear long ago that if anomalous nucleosides were to act on nucleic acid biosynthetic pathways, probably at the nucleotide level, one should be concerned with catabolic pathways which such nucleosides might take in biological systems. A partial representation of this problem is shown in *Figure 2.* Cytidine nucleosides both of the ribo or 2'-deoxyribo type can be converted by nucleoside deaminases to their uracil counterparts, a matter of importance as we shall see later. Uridine type nucleosides may undergo glycosyl cleavage at the C'l—Nl bond by nucleoside phosphorylases to produce the free aglycon, the latter of which may undergo catabolic or anabolic steps.

Several years ago we embarked on a programme to synthesize 1-β-D-aldopentofuranosyl pyrimidine nucleosides with configurational alterations in the sugar moiety. A representation of these isomers is shown in *Figure 3.* It became quite apparent as a result of studies in our own and in Cohen's laboratory that the susceptibility of 1-β-D-aldopentofuranosyl pyrimidines to cleavage or deamination by crude extracts of *E. coli* was highly influenced by the configuration of the sugar moiety[7, 8]. Pyrimidine nucleosides of the lyxo, xylo or arabino configuration were poor substrates for pyrimidine phosphorylases from *E. coli* extracts under conditions which would readily

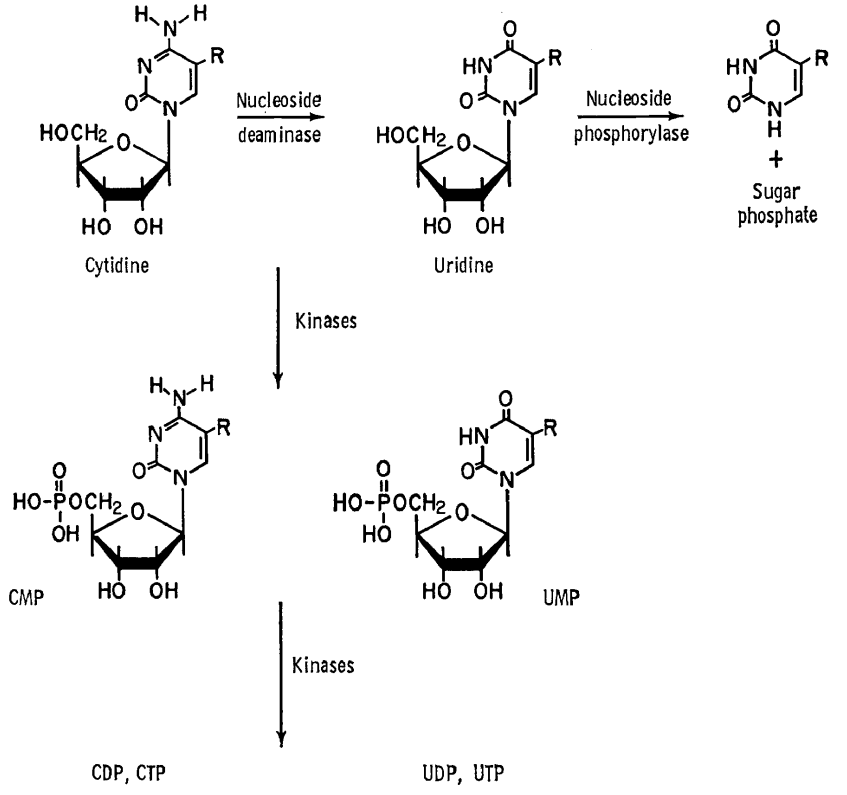


Figure 2. A partial representation of the metabolic fate of pyrimidine ribonucleosides.

cleave ribosylthymine or uridine. A rather similar pattern was observed with the cytosine analogues, that is, the lyxo and xylo analogues were inert toward deaminases while the arabino isomer was deaminated by *E. coli* but at a slower rate than was cytidine or 2′-deoxycytidine.

Some of these considerations were involved in our studies with 5-fluoropyrimidine nucleosides. 5-Fluoro-2′-deoxyuridine (FUDR), first prepared by Duschinsky and Heidelberger, has acquired considerable importance as a cancerostatic drug[9]. From the studies of Cohen[10] and of Heidelberger[9] it is generally accepted that the anti-tumor activity of 5-fluoro-2′-deoxyuridine (or of 5-fluorouracil) involves the enzymatic conversion of these drugs to 5-fluoro-2′-deoxyuridine 5′-phosphate (*Figure 4*) which blocks the biosynthesis of DNA thymine by inhibition of the enzymatic "methylation" of deoxyuridylic acid to thymidylic acid by thymidylate synthetase. However, the efficacy of 5-fluoro-2′-deoxyuridine may be weakened by catabolic processes (glycosyl cleavage to 5-fluorouracil and to catabolic products derived therefrom). As we have mentioned, arabinosyluracil and arabinosylthymine are virtually resistant to cleavage by nucleoside phosphorylases in cell free systems. Moreover, since Pizer and Cohn[11] had shown that 1-β-D-arabinosyluracil is phosphorylated enzymatically to its 5′-nucleotide which

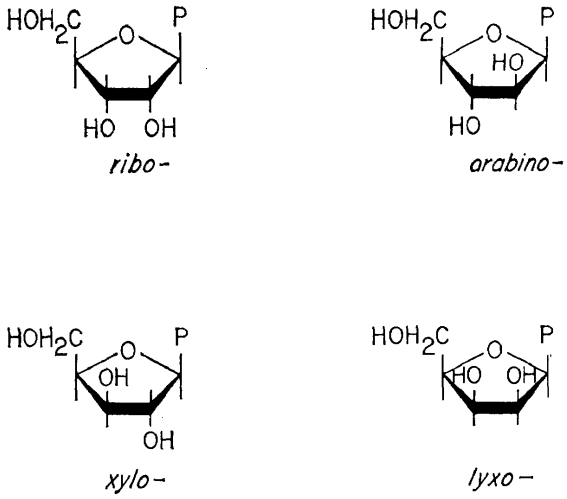


Figure 3. The four 1-β-D-aldopentofuranosyl pyrimidines. (P = thymine, uracil or cytosine)

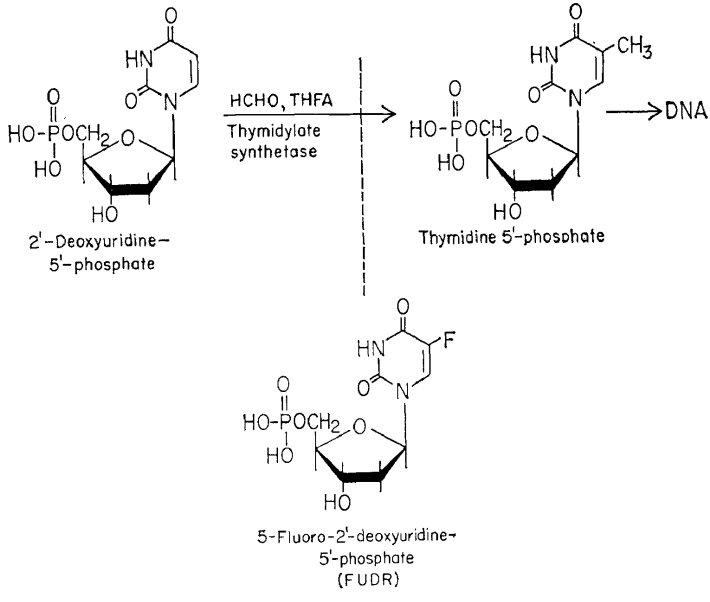


Figure 4. 5-Fluoro-2′-deoxyuridylic acid as a blocking agent in the "de novo" synthesis of DNA thymine[9, 10]

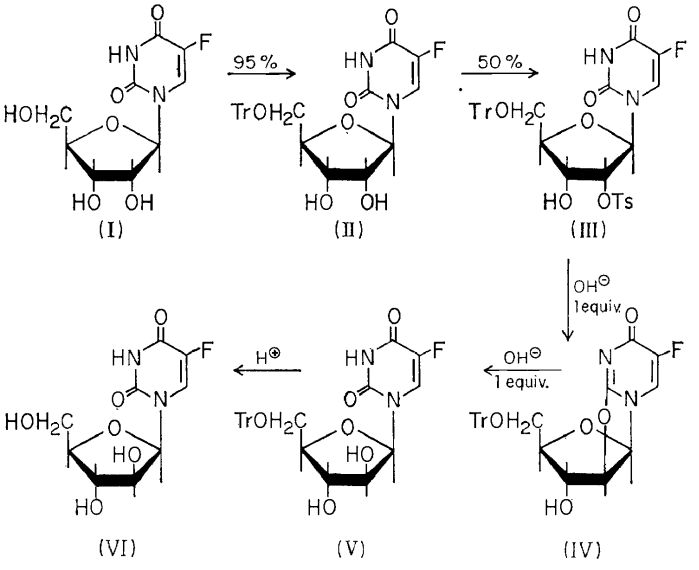


Figure 5. A synthesis of Ara-FU from 5-fluorouridine[12]

can then partake (albeit poorly) in the "methylation" step, the synthesis of 1-β-D-arabinofuranosyl-5-fluorouracil (Ara-FU) was undertaken with the hope that it might be chemotherapeutically active and preserved longer *in vivo* than 5-fluoro-2′-deoxyuridine.

The synthesis of Ara-FU from 5-fluorouridine, shown in *Figure 5*[12], is a standard example by which a ribonucleoside (I) is converted to an arabino analogue (VI) *via* an anhydro nucleoside intermediate (IV). Except for the *p*-tosylation step (II → III) which proceeds in ~ 50 per cent yield (some 2′,3′-di-tosylate is also formed), the overall yields are good. More recently, we have developed methods for 2,2′-anhydro nucleoside formation which are superior (*vide infra*).

Ara-FU (VI) showed as good a chemotherapeutic index against leukemia B82 in mice as did 5-fluoro-2′-deoxyuridine though about four-fold higher concentrations of VI were needed to produce this effect[12].

Note that two successive treatments with alkali are given to convert the monotosylate III to V. During a large scale preparation of Ara-FU, we attempted to convert III with an *excess* of alkali to V. The yield was very poor. Closer examination of this reaction led to new and rather exciting nucleoside chemistry which will be discussed later.

An interesting reaction is the general, facile conversion of *ribo* pyrimidine nucleosides to their *lyxo* analogues[12, 13] (*Figure 6*). For example, mesylation of 5′-*O*-trityl-5-fluorouridine (II) gave compound VII which was de-tritylated to the di-mesylate (VIII). When VIII was refluxed in water, 1-β-D-lyxofuranosyl-5-fluorouracil was obtained[12]. Thus, in one reaction, the configuration of the sugar moiety was epimerized at *both* the 2′ and 3′ position [from ribo (*cis*) to lyxo (*cis*)]. Detailed studies[21, 13] of the course of this unexpected reaction in refluxing water are summarized in *Figure 7*.

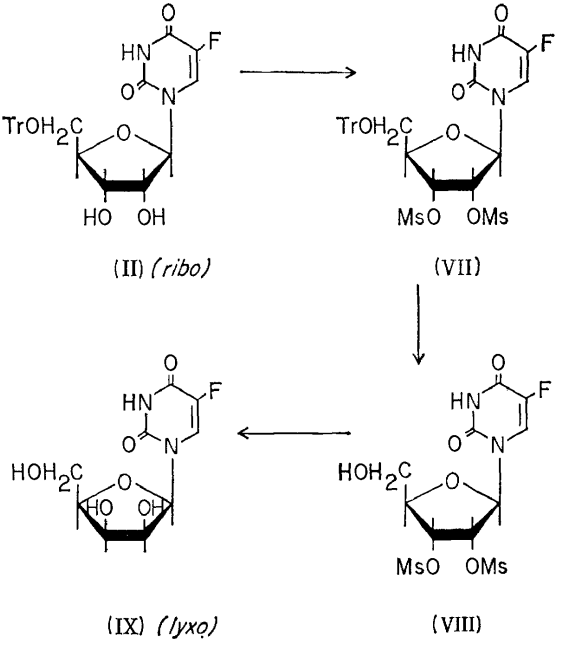


Figure 6. A facile conversion of *ribo* to *lyxo* nucleosides[12, 13]

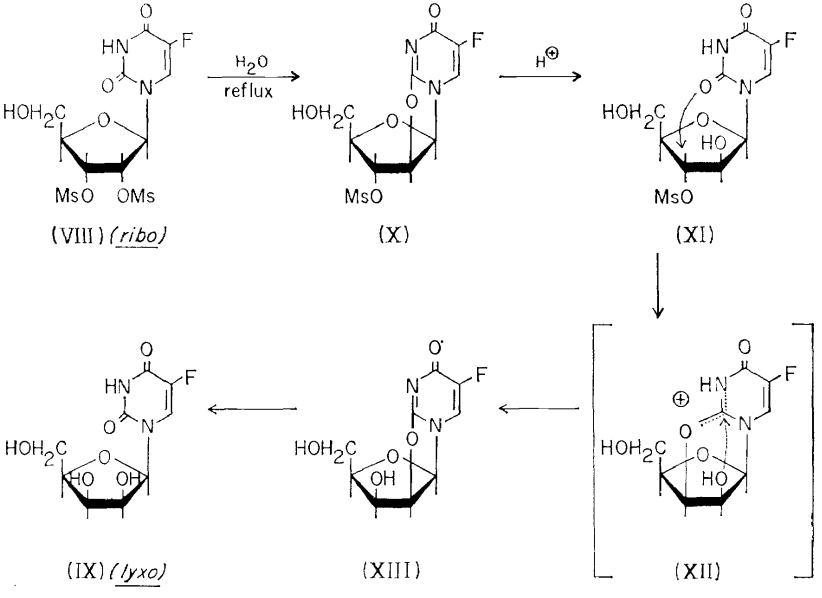


Figure 7. Detailed steps in the conversion of *ribo* to *lyxo* nucleosides[12, 13]

The first step is the formation of a 2,2′-anhydro-*arabino* nucleoside X (with the liberation of methanesulphonic acid) which under acid catalysis is cleaved to XI. Nucleophilic attack by the 2-carbonyl of XI on C-3′ gives the 2,3′-anhydro-*lyxo* nucleoside (XII) which is converted to the 2,2′-anhydro-*lyxo* nucleoside XIII by attack of the "up" 2′-hydroxyl on a carbonium ion generated at C-2 of XII. Finally, anhydro nucleoside XIII is hydrolyzed to the lyxosyl nucleoside (IX). In this sequence of reactions only intermediate XII is postulated. Experimental evidence has been obtained for all the other intermediates. Incidentally, the fluoro-*lyxo* nucleoside (IX), unlike its arabino analogue (VI), shows no activity against leukemia B82 in the mouse.

The conversion of a *ribo* nucleoside to its *xylo* epimer (*Figure 8*)[14] is best

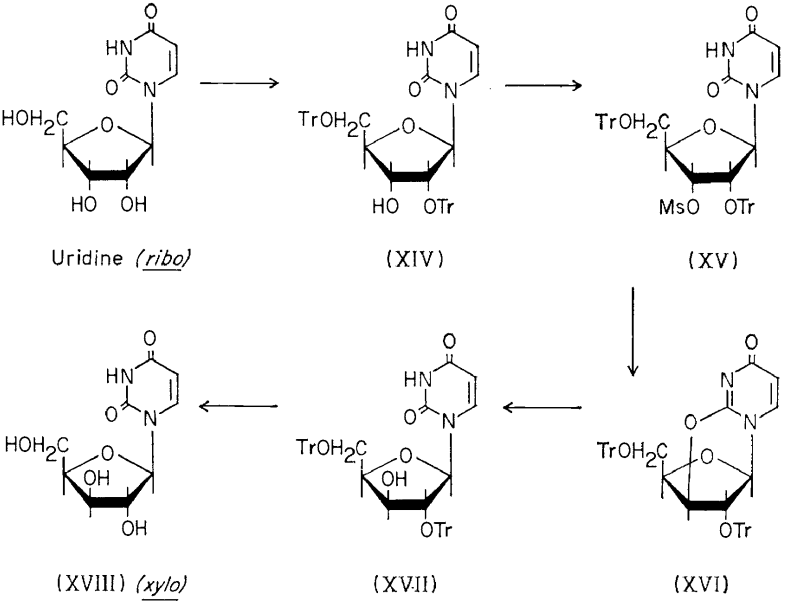


Figure 8. Conversion of *ribo* to *xylo* uracil[14]

achieved from a suitably-protected 3′-sulphonylated uridine. Such derivatives can now be easily obtained by tritylation of uridine with an excess of trityl chloride to give a fair yield of a crystalline product which was shown to be 2′,5′-di-*O*-trityluridine (XIV). Mesylation of XIV gave 3′-*O*-mesyl-2′,5′-di-*O*-trityluridine (XV). Treatment of XV with sodium benzoate in DMF afforded the 2,3′-anhydro-*xylo* nucleoside (XVI) which, after treatment with alkali followed by de-tritylation, gave 1-β-D-xylofuranosyluracil (XVIII) as the sole product[14].

Anhydronucleosides may undergo rearrangements *in situ*, especially when nucleophilic substituents on the sugar moiety are favourably located. An example[14] of this aspect of anhydronucleoside chemistry is shown in *Figure 9*. 3′-*O*-Mesyluridine (XIX) was prepared by de-tritylation of XV and ben-

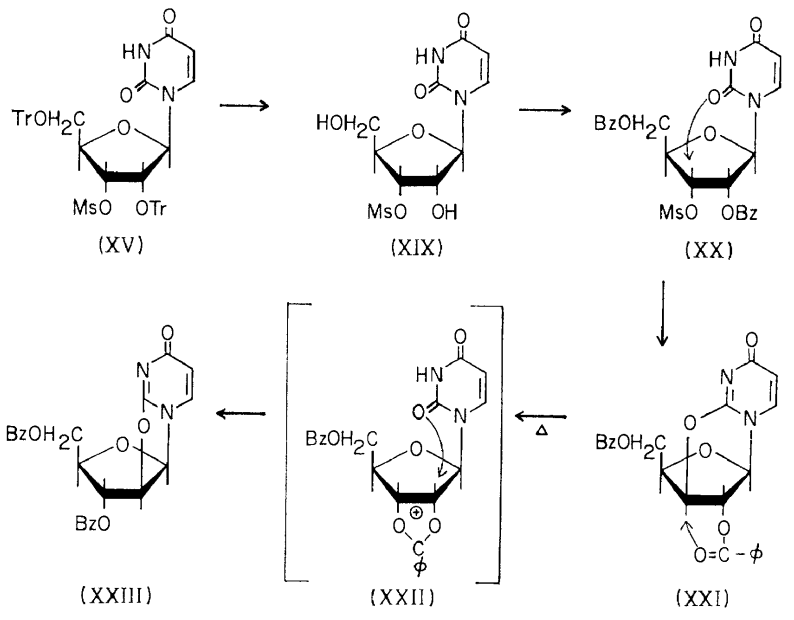


Figure 9. Rearrangement of a 2,3′-anhydronucleoside to a 2,2′-isomer via an orthoester ion[14]

zoylated to 3′,5′-di-*O*-benzoyl-3′-*O*-mesyluridine (XX). Treatment of XX with sodium benzoate in DMF gave the 2,3′-anhydro-*xylo*-nucleoside (XXI). (Compound XXI was also obtained by direct benzoylation of 2,3′-anhydro-xylosyluracil). When XXI was heated in a melting point tube, it melted and resolidified. The resolidified product was the 2,2′-anhydro-*arabino*-nucleoside (XXIII). Thus, in one step, anhydro bond and benzoyl migration occurred. A most plausible mechanism for this rearrangement is obviously neighbouring group participation by attack of the 2′-benzoate of XXI on C-3′ to give a transient orthoester ion (XXII) which then undergoes attack by the 2-carbonyl on C-2′ to give the 2,2′-anhydro-arabino nucleoside (XXIII).

Anhydronucleosides are also useful for the stereochemically-controlled introduction of functional groups into the secondary positions of the sugar moiety. The earliest example is the preparation (*Figure 10*) of a 3′-halogeno-3′-deoxythymidine (XXV) by Michelson and Todd[15] by treatment of the 3′-mesylate (XXIV) with lithium bromide or sodium iodide in acetone at 100°. They proposed that the 3′-halogeno substituent was in the "down" or *ribo* form since XXV could be converted into the 2,3′-anhydro derivative (XXVI). Consistent with their experiments is the reasonable assumption that an anhydro nucleoside intermediate was involved in the conversion of XXIV→ XXV.

We encountered a somewhat similar problem in our studies[16] with di-*O*-mesylthymidine (XXVII) which is easily obtained by mesylation of thymidine (*Figure 11*). Treatment of the di-mesylate with excess sodium benzoate in DMF for 10 hours at reflux temperature gave a high yield of 3′,5′-di-

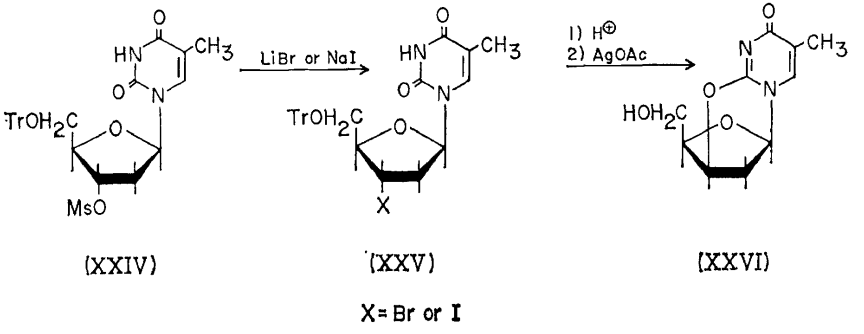


Figure 10. Synthesis of 3′-deoxy-3′-halogenothymidine by Michelson and Todd[15]

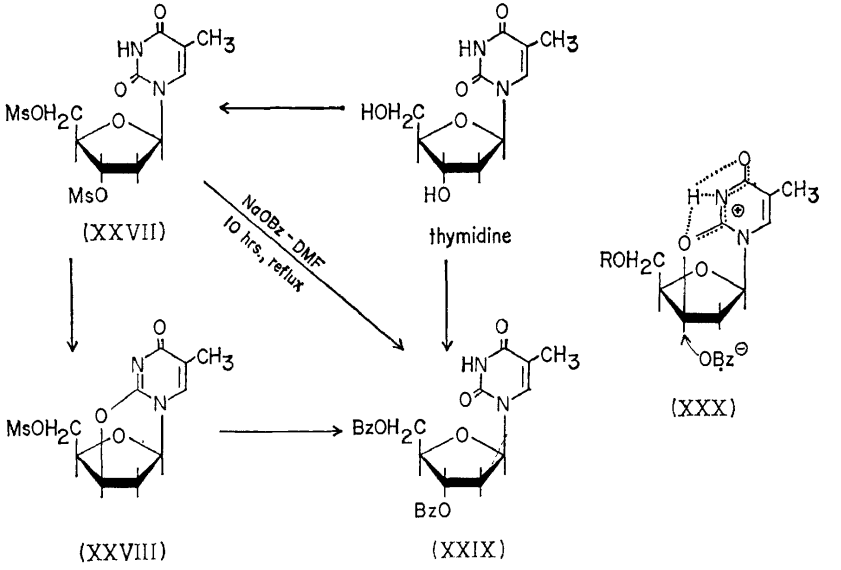


Figure 11. Reactions of 3′,5′-di-*O*-mesylthymidine[16]

O-benzoyl-thymidine. This dibenzoate was also obtained by direct benzoylation of thymidine, which established the fact that, in the conversion of the dimesylate XXVII to the dibenzoate XXIX, no *net* inversion had occurred. Here, too, it is reasonable to postulate a double Walden inversion at C-3′ of XXVII involving first the formation of anhydronucleoside XXVIII (displacement of the 3′-mesylate) followed by attack of benzoate ion at C-3′ and then at C-5′ to give di-*O*-benzoylthymidine. Indeed, when this reaction (XXVII → XXIX) was performed under milder conditions (0·2 hours

at 95°), anhydronucleoside XXVIII was actually isolated from the reaction mixture. It should be noted that a similar pattern is observed when 5′-*O*-trityl-3′-*O*-mesylthymidine is treated with sodium benzoate in DMF under similar conditions. Yet, when we tried to convert the anhydronucleoside XXVIII to XXIX under *identical* reaction conditions the yield was very poor. Similarly poor yields were obtained when the 5′-*O*-trityl analogue of XXVIII was employed.

In the *overall* conversion of the dimesylate to the dibenzoate with excess sodium benzoate, two equivalents of mesylate are liberated. Formation of the 2,3′-anhydronucleoside by attack of the 2-carbonyl on C-3′ requires the concomitant generation of benzoic acid in the reaction milieux, and this acid was present during the overall conversion of XXVII to XXIX. The formation of benzoic acid would not occur when anhydronucleoside (XXVIII) is treated with excess sodium benzoate in DMF. However, when benzoic acid was *added* to the reaction of anhydronucleoside XXVIII in excess sodium benzoate–DMF, a high yield of XXIX was obtained. A plausible explanation of this catalysis is that the benzoic acid (liberated *in situ* in the reaction of XXVII or *added* to the reaction of XXVIII) protonates the conjugated system of the aglycon of anhydronucleoside XXVIII (see XXX) and thus renders the C—3′ position more susceptible to nucleophilic attack by benzoate ion. An essentially similar mechanism was implied by Murdock and Angier[17] for the formation of halogenated 1-cyclopentane isosteres of thymidine from an "anhydronucleoside" intermediate.

These studies suggested that under acid-catalyzed conditions other nucleophiles may be introduced into the *ribo* or "down" configuration of the sugar portion of anhydronucleosides or their sulphonyloxy precursors. Application ot this rationale to the synthesis of anomalous thymidine and deoxycytidine type nucleosides is shown in *Figure 12*[16]. Reaction of anhydronucleoside XXXII with potassium thiobenzoate in DMF *with added benzoic acid* gave the 3′-deoxy-3′-*S*-thiobenzoate which was detritylated and deesterified to 3′-thiothymidine (XXXIV, isolated as the disulphide). Similarly, treatment of the anhydronucleoside (XXXII) with potassium phthalimide in DMF *with added phthalimide* gave a high yield of the 5′-*O*-trityl-3′-phthalimido derivative XXXV. The same product may be obtained directly by treatment of the 3′-mesylate (XXXI) with potassium phthalimide in DMF without the addition of phthalimide to the reaction. "3′-Aminothymidine" is readily obtained by de-tritylation and deacylation of XXXV. The 4-thione analogue (XXXVII) may be prepared from XXXVI by acetylation and thiation with phosphorus pentasulphide in pyridine. Treatment of thione (XXXVII) with alcoholic ammonia gave the 3′-phthalimido derivative XXXVIII. Deacylation of the latter compound with *n*-butylamine at 105° for 20 hours gave, unexpectedly, a mixture of 3′-amino-2′,3′-dideoxy-5-methylcytidine as well as the N^4-butyl analogue XXXIX from which the former was isolated in crystalline form. We have since shown that 2′-deoxycytidine is also converted to N^4-butyl-2′-deoxycytidine under similar conditions which suggests that such "amine-exchange" reactions are rather general for cytosine nucleosides.

Anhydronucleosides (under acid catalyzed conditions) are useful for the introduction of halogens into the sugar moiety (*Figure 13*). Treatment of

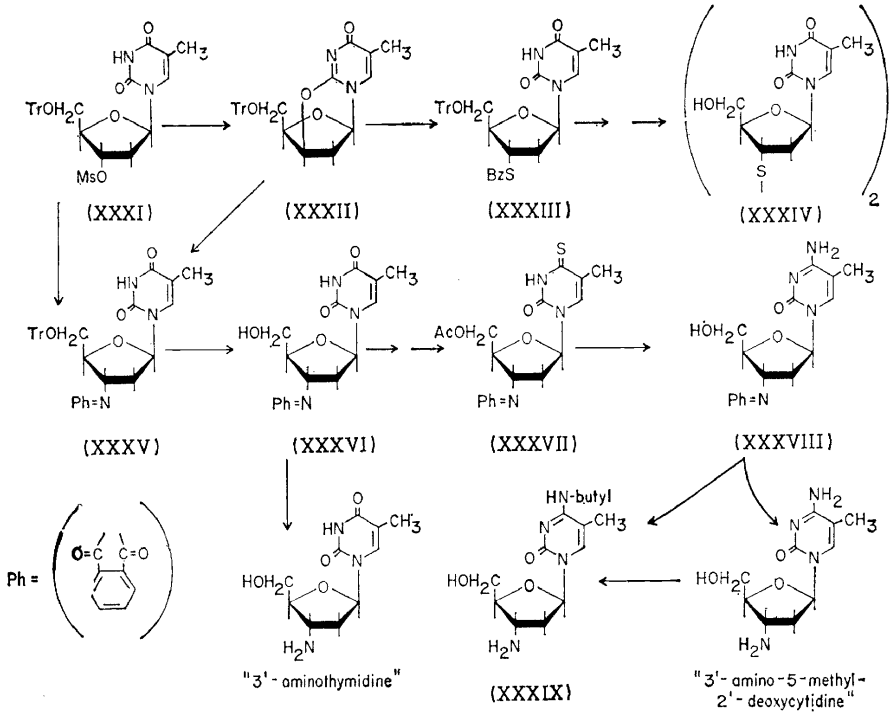


Figure 12. 3′-Substituted-3′-deoxy-thymidines and -5-methylcytidines[16]

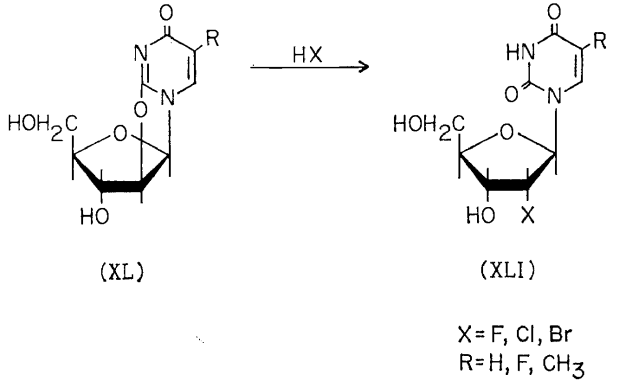


Figure 13. 2′-Halogeno-2′-deoxyuridines[18]

2,2′-anhydronucleosides (XL) with anhydrous hydrogen halides (HBr, HCl, HF) gave good yields of the 2′-halogeno-2′-deoxy nucleosides (XLI)[18]. It should be noted that Brown and associates[6] had previously shown that 5′-*O*-acetyl-2′-*O*-tosyluridine may be converted into 2′-iodo-2′-deoxyuridine by reaction with sodium iodide in acetonylacetone and showed that a 2,2′-

anhydronucleoside was an intermediate. Their reaction may also be viewed as an example of acid catalysis (protonation of the anhydro intermediate by the acid liberated *in situ* followed by attack by iodide ion on C-2′ of the sugar moiety). A similar explanation would hold for the synthesis by Michelson and Todd (*Figure 10*) of 3′-halogenothymidines XXV from XXIV.

Anhydronucleosides may also be utilized as chemical precursors for the synthesis of nucleoside epoxides (*Figure 14*). Treatment of tri-*O*-mesyluridine

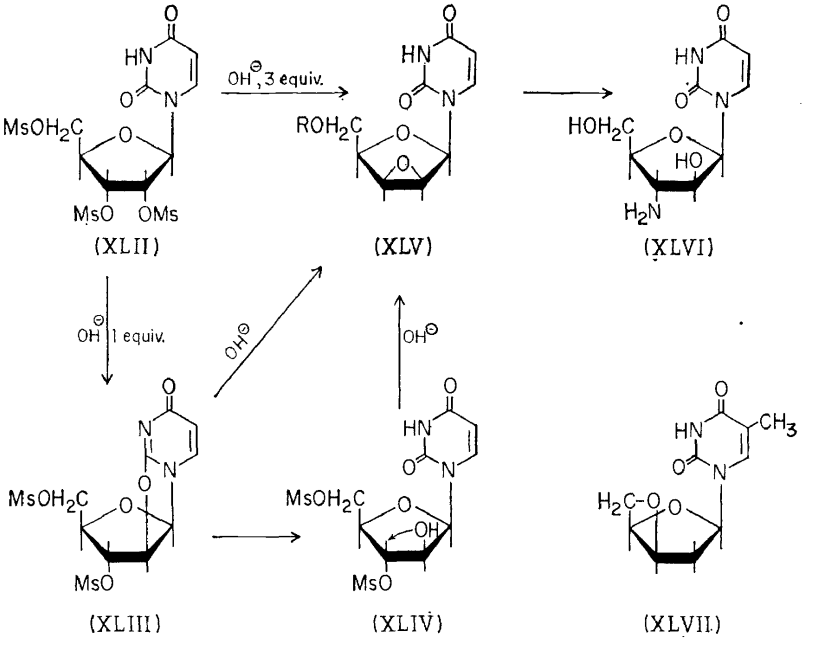


Figure 14. Nucleoside epoxides[19, 20]

(XLII) with three equivalents of alkali gave good yields of the 2′,3′-epoxide (XLV, R = mesyl)[19]. This conversion most likely proceeds *via* the anhydronucleoside (XLIII) and the arabino nucleoside (XLIV) since either of the latter two derivatives are converted to epoxide XLV by alkali. Epoxide XLV (R = OH) has been converted into 1-(3-amino-3-deoxy-β-D-arabinofuranosyl)uracil (XLVI) by reaction with alcoholic ammonia at elevated temperature[19]. The 3′,5′-epoxide of 2′-deoxy-lyxosylthymine (XLVII) has been synthesized by treatment of the 2,3′-anhydro derivative (XXVII, see *Figure 11*) with aqueous alkali[20]. In this case also, epoxide formation is the result of anhydro bond cleavage followed by the displacement of the mesylate group by attack of a neighbouring sugar hydroxyl anion. The preparation of a host of 2′,5′- and 3′,5′-epoxides of aldopentofuranosyluracils has been reported[13b, 21].

In the course of hydrolytic studies on the 5′-iodo or 5′-mesyloxy derivatives (*Figure 15*, XLVIII) of arabinosyluracil, evidence was obtained[21] for intramolecular transformations involving anhydronucleosides. Treatment

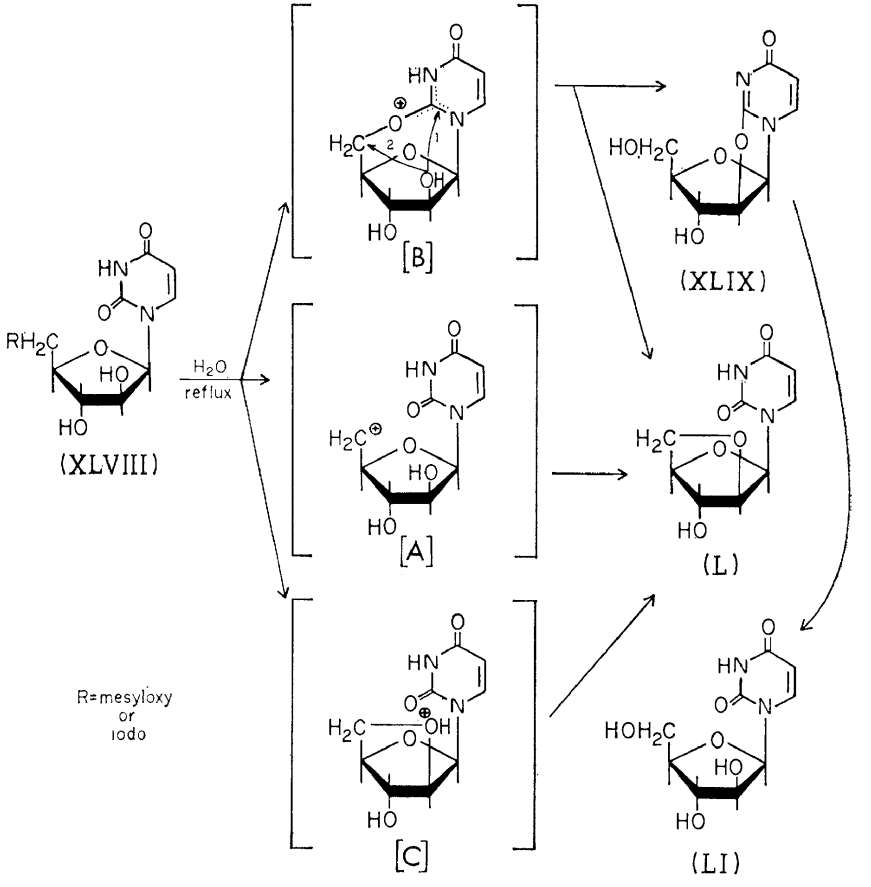


Figure 15. Some intramolecular transformations involving anhydronucleosides[21]

of XLVIII (R = mesyloxy or iodo) in boiling water yielded three products: the 2,2′-anhydro nucleoside (XLIX), the 2′,5′-epoxy nucleoside (L) and arabinosyluracil (LI). A plausible explanation of these displacements of the C-5′ leaving group of XLVIII is one which involves ions (A ⇋ B ⇋ C) as intermediates. Attack by the 2-carbonyl of the aglycon on C-5′ would give the 2,5′-anhydronucleoside, intermediate (B). Evidence in favour of (B) is the isolation of XLIX. The mechanism of 2,2′-anhydronucleoside formation may be visualized as an intramolecular attack by the 2′-hydroxyl group on the electrophilic pyrimidinyl C-2 site of the 2,5′-anhydro resonance hybrid (B) (arrowed pathway 1, *Figure 15*). If elimination of the C-5′ leaving group of XLVIII occurs *without* participation of neighbouring groups, then carbonium ion (A) would form which probably could then be converted to cations (B) and (C) by nucleophilic attack of the 2-carbonyl or the 2′-hydroxyl on C-5′. The formation of L could occur by attack of the 2′-hydroxyl of [A] on C-5′. The formation of arabinosyluracil (LI) from

XLVIII in this hydrolysis may result from numerous pathways. Intermediates (A, B, C) may react at their respective electrophilic sites with water to give LI. Also, LI may form by hydrolysis of the 2,2′-anhydro nucleoside XLIX.

The alterations produced in the sugar moiety via anhydronucleoside intermediates described thus far have been brought about by the use of *inter* or *intra* nucleophilic substitution reactions. Recently, Horwitz and coworkers[22] demonstrated that certain anhydronucleosides may be employed for the introduction of 2′,3′-unsaturation in the carbohydrate moiety via base-catalyzed elimination reactions (*Figure 16*). Treatment of

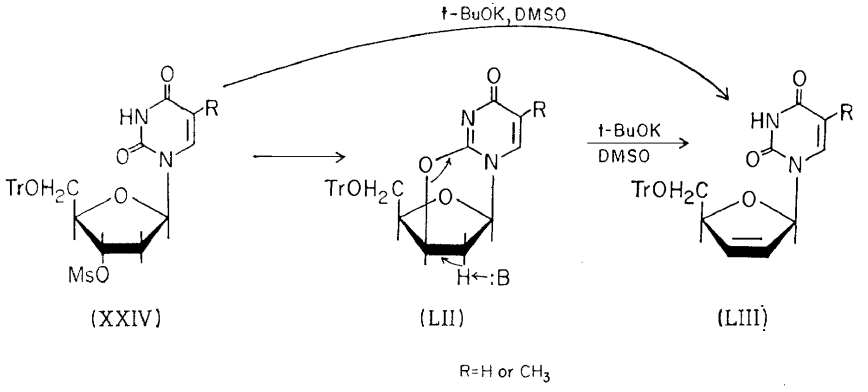


Figure 16. Synthesis of 2′,3′-unsaturated uridines by Horwitz *et al.*[22]

anhydronucleoside LII with potassium *tert*-butoxide in dimethyl sulphoxide gave good yields of the 2′,3′-ene nucleoside LIII by α-proton abstraction at C-2′. Similarly, treatment of the 3′-*O*-mesyl precursor (XXIV) directly with potassium *tert*-butoxide in DMSO also produced the 2′,3′-unsaturated nucleoside (LIII). Obviously, this latter reaction (XXIV→ LIII) proceeded through anhydronucleoside LII. Detritylation of nucleosides (LIII) gave the corresponding "thymidinene" and "uridinene"[22].

The discussion thus far has demonstrated amply that pyrimidine anhydronucleosides are versatile intermediates and are useful for (a) the alteration of configuration and, (b) the introduction of functional groups into the sugar moiety. We will now consider the use of anhydronucleosides to introduce modifications in the aglycon—including rearrangements of the heterocyclic moiety. Our recent chemical studies in this area were partially motivated by the discovery[23] that 1-β-D-arabinofuranosylcytosine (Ara-C) had shown useful activity in the clinical treatment of acute leukemia and lymphomas, but pharmacological studies in man and mouse demonstrated that this nucleoside is rapidly deaminated to the inactive metabolite, 1-β-D-arabinosyluracil (Ara-U).

The introduction of functional groups into the heterocyclic moiety of nucleosides by way of anhydronucleoside intermediates was first achieved by Brown and coworkers[24]. They demonstrated (*Figure 17*) that treatment of the 2,5′-anhydronucleoside (LIV) with methanolic ammonia for 5 days

yielded the isocytidine derivative (LVI) by way of the 2-methoxy nucleoside (LV). Under similar reaction conditions they also prepared 1-β-D-arabinosylisocytosine from 2,2′-anhydro-arabinosyluracil.

In the course of our studies with 2′-halogeno uridines we investigated the synthesis (*Figure 18*) of 2′-halogeno-2′-deoxycytidines (LVIII)[25]. Treat-

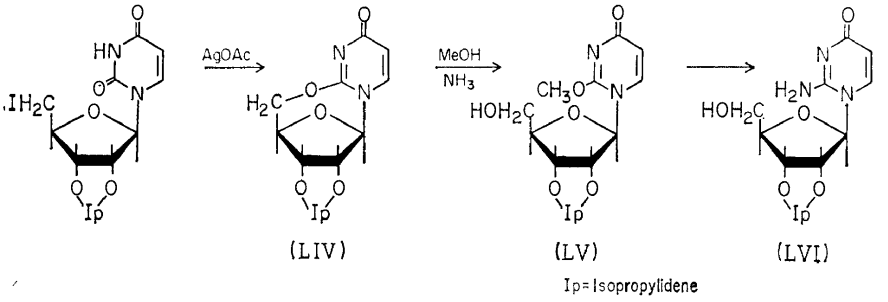


Figure 17. Synthesis of isocytidine from a 2,5′-anhydrouridine by Brown *et al.*[24]

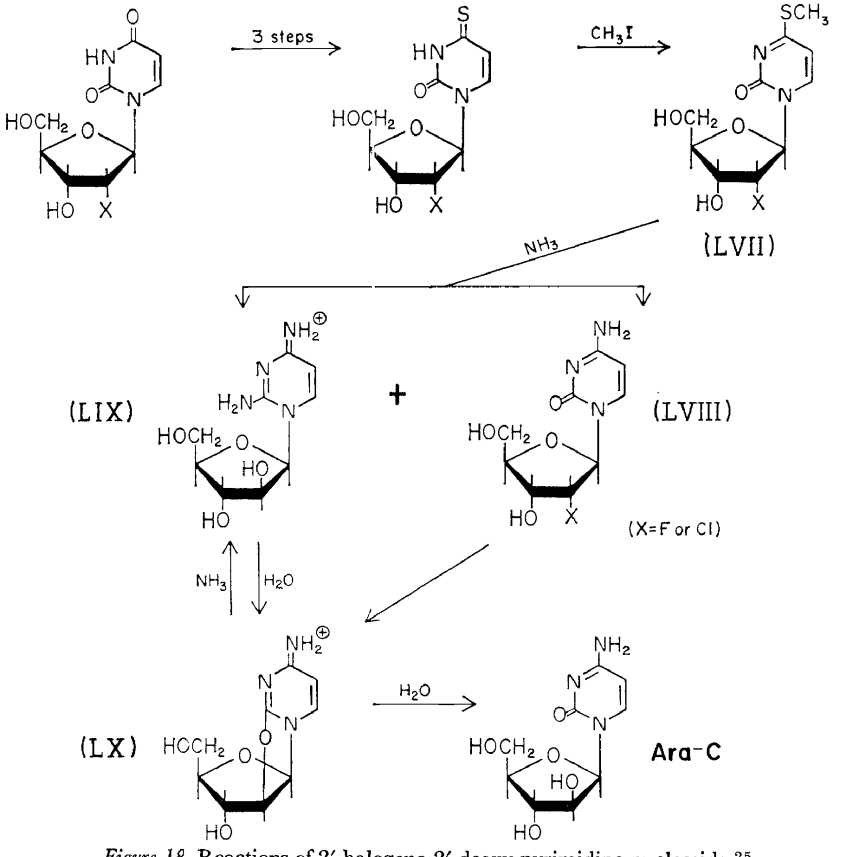


Figure 18. Reactions of 2′-halogeno-2′-deoxy-pyrimidine nucleosides[25]

ment of the 4-methylmercapto-2′-halogeno nucleoside LVII with liquid ammonia at 60° for several days gave a mixture of the desired 2′-halogenocytidine LVIII plus the "2-amino-4-imino" nucleoside halide salt LIX. When this mixture was boiled in anhydrous dioxane, the 2′-halogeno derivative was converted to the halide salt of 2,2′-anhydro-arabinosylcytosine LX. (This 2,2′-anhydronucleoside salt (as well as Ara-C) had been synthesized previously by Walwick *et al.*[26], by another route). Attempts to neutralize the HCl salt of LX converted it rapidly to Ara-C. Anhydronucleoside LX was also converted in liquid ammonia to the amino-imino nucleoside LIX. Compound LIX was hydrolyzed in dilute alkali to Ara-C.

These conversions and related hydrolytic experiments[25] indicate that the formation of the "amino-imino" nucleoside (LIX) from LVII probably occurred via the 2′-halogenocytidine LVIII and anhydronucleoside LX under the ammonolysis conditions employed. The conversion of LIX to LX is readily explained by attack of the 2′-hydroxyl on C-2. These experiments suggest that the diaminopyrimidine nucleoside (LIX) and the anhydronucleoside (LX) may serve as "masked" Ara-C precursors *in vivo*. Preliminary studies[27] at our Institute showed that both LIX and LX produced increases in the life span of leukemic mice.

The mode of action of Ara-C is not firmly established[8]. One mechanism of action of Ara-C on the nucleotide level (*Figure 19*) has been suggested by Chu and Fischer[28] based on their studies with mouse leukemia 5178Y cells. According to their mechanism, Ara-C is anabolized to Ara-CMP and further to an intermediate (probably Ara-CDP) which blocks the enzymatic conversion of CDP→ dCDP, thus blocking DNA biosynthesis. (The action of Ara-C against several types of experimental leukemias is reversed by 2′-deoxycytidine). As mentioned previously, the efficacy of Ara-C as a chemotherapeutic agent is considerably diminished by its rapid *in vivo* deamination (*Figure 20*) to Ara-U. This posed some interesting questions. For example, what would be the chemotherapeutic effect of the 5-fluoro analogue (Ara-FC)? Would it behave like Ara-C [cytidylate reductase (?) inhibitor] or would it block thymidylate synthetase as do the 5-fluoropyrimidines? Further, if some of the Ara-FC underwent enzymatic deamination *in vivo*, Ara-FU would be formed. It will be recalled that Ara-FU has shown activity[12, 29] against mouse leukemia B82 and Sarcoma 180. Alternatively, could chemotherapeutically-active analogues of Ara-C be synthesized which would not undergo enzymatic deamination *in vivo*? Conversely, would some of these derivatives inhibit deoxycytidine deaminase and, if so, would the anti-leukemic activity of Ara-C be enhanced by combined chemotherapy with such inhibitors? We were encouraged by preliminary studies by Camiener[23i] showing that the known compounds[30], 5-methyl-2′-deoxycytidine and its 4-hydroxylamino analogue, inhibited human liver deoxycytidine deaminase *in vitro*. We therefore undertook the synthesis of Ara-FC by a procedure which would also give access to other 4-substituted analogues. These studies led to a new method for 2,2′-anhydronucleoside formation and, eventually, to a general involvement in the use of anhydronucleosides for rearrangement of the heterocyclic moiety of nucleosides.

The synthesis of Ara-FC and related nucleosides is given in *Figure 21*[29]. We had found[31a] that reaction of 5′-*O*-trityluridine (LXI, R = H) with

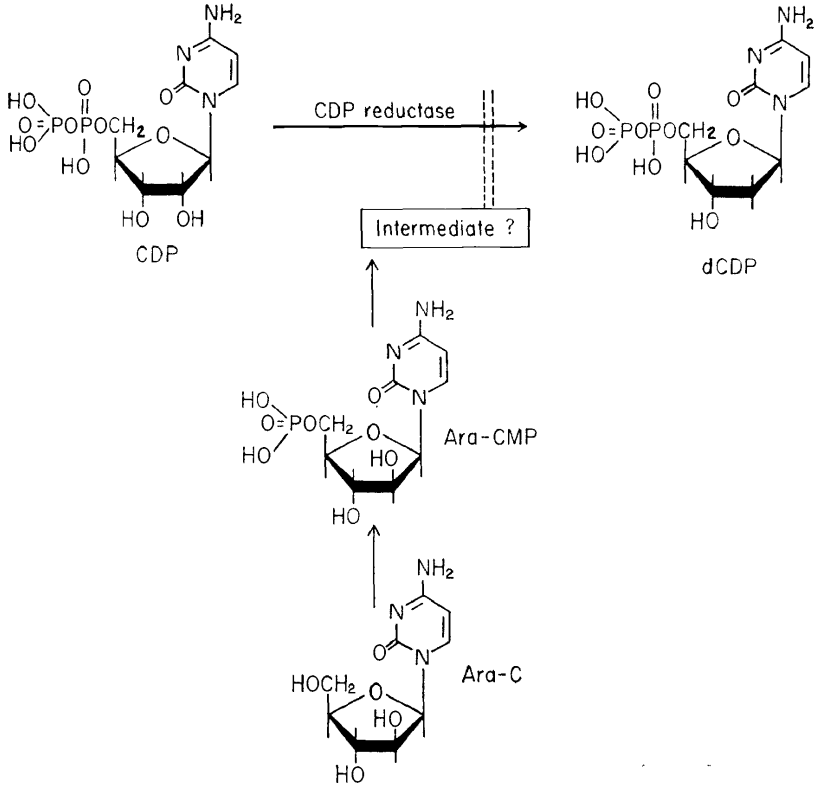


Figure 19. A mechanism of action of Ara-C as proposed by Chu and Fischer[28]

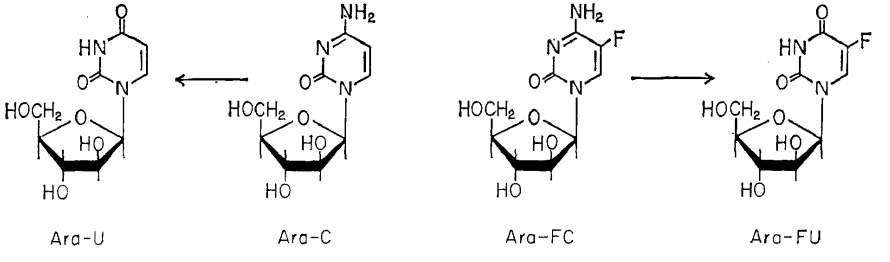


Figure 20.

thiocarbonyldiimidazole in hot toluene gave *directly* high yields of the 2,2′-anhydronucleoside derivative (LXIII, R = H, R′ = trityl). When uridine itself was used in this reaction, a fair yield of the free 2,2′-anhydronucleoside (LXIII, R,R′ = H) was obtained. Similar reactions were carried out with various 5-substituted uridines (R = trityl, R′ = CH_3,F) and there is little doubt that this general method is the simplest approach as of this date to the

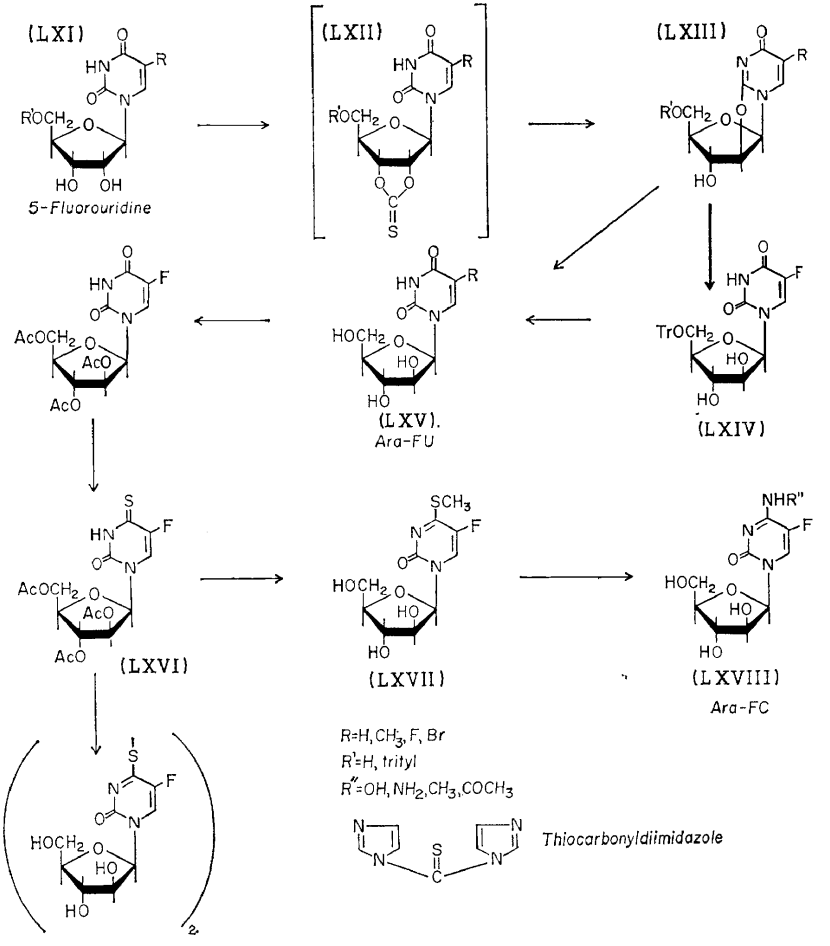
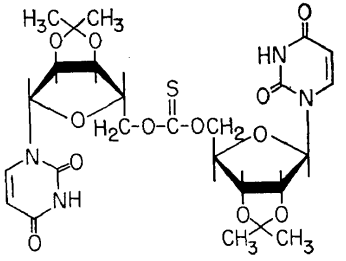


Figure 21. Synthesis of Ara-FU, Ara-FC and related nucleosides[29, 31a]

conversion of *ribo* to *arabino* pyrimidine nucleosides. We postulated[31a] the 2',3'-thionocarbonate (LXII) as an intermediate in this facile conversion and soon thereafter, Ruyle and coworkers[31b] isolated LXII in high yield by conducting this reaction in tetrahydrofuran at room temperature. More recently, Hampton *et al.*[31c] achieved, by a similar principle, the conversion of uridine to LXIII in good yield by use of diphenylcarbonate as the "anhydro-forming" reagent. Incidentally, reaction of 2',3'-*O*-isopropylideneuridine with thiocarbonyl-diimidazole affords the isopropylidene derivative of the di-nucleoside, uridylyl-(5'→5')-uridine thionocarbonate[32] (compound A).

Though anhydronucleosides LXIII may be converted directly to LXV by alkaline treatment and detritylation, when the 5-position bears an electronegative group such as fluorine, care must be taken to avoid excess alkali for



Compound A

long periods for reasons to be discussed later. Thus, treatment of anhydronucleoside (LXIII, R = F) with a slight excess of alkali followed by neutralization of the reaction with acetic acid gave the *arabino* derivative LXIV which was detritylated in acid to Ara-FU.

The conversion of Ara-U to Ara-C was achieved by use of the thiation procedure[30] devised for the conversion of uridine-type nucleosides to their cytosine analogues. Acetylation and thiation of LXV gave the 4-thio nucleoside LXVI which was S-methylated to LXVII and then converted to Ara-FC[29] by reaction with liquid ammonia. Reaction of the S-methyl nucleoside LXVII with other nucleophiles gave the corresponding 4-hydrazino, 4-hydroxylamino, and the 4-methylamino derivatives (LXVIII, R″ = NH_2,OH,CH_3)[33]. By generally similar procedures, we also prepared the 4-hydrazino, 4-hydroxylamino and 4-methylamino analogues of Ara-C.

Ara-FC possesses a high degree of chemotherapeutic activity against transplanted mouse leukemia[34a]. It is more active on a molar basis than Ara-C, 5-fluorouracil or 5-fluoro-2′-deoxyuridine against leukemia P815 and somewhat more active than Ara-C in Leukemia P388. In mouse leukemia L1210, the activity of Ara-FC and Ara-C are approximately equal. Both drugs are equally inhibitory against cells of leukemias P815Y and P388SK in tissue culture. These inhibitory effects are blocked by 2′-deoxycytidine but not by thymidine. These and other data suggest that the mechanism of action of Ara-FC is probably akin to that for Ara-C, rather than to that of 5-fluorouracil or the other 5-fluorinated nucleosides. It is concluded that Ara-FC is somewhat more active than Ara-C and merits clinical trials[34a]. Preliminary experiments (by Dr. E. Grunberg of Hoffmann-La Roche, Inc., Nutley) indicated that Ara-FC, like Ara-C and 5-iodo-2′-deoxyuridine, exhibits essentially similar anti-viral activity in preventing the development of herpes keratitis in rabbits[29].

A detailed study[34b] of the N^4-substituted derivatives of Ara-FC (LXVIII) and the corresponding Ara-C derivatives showed that none of these were deaminated *in vitro* by human liver or mouse kidney homogenates which easily deaminate Ara-C and Ara-FC. Both Ara-C and Ara-FC are deaminated by the high content[23g] of deoxycytidine deaminase present in these systems. These data[34b] attest to the high degree of specificity which deoxycytidine deaminase in these systems has with respect to alterations in the exocyclic amino function. The N^4-hydroxy analogues of Ara-C and Ara-FC are also active against mouse leukemias (including a 5-fluorouracil resistant line) and these effects, too, are reversed by deoxycytidine. The N^4-methyl

and N^4-hydrazino derivatives of Ara-C and Ara-FC are inactive in mouse leukemia and in Burkitt's cell culture.

The N^4-hydroxy and N^4-methyl derivatives of Ara-C (LXVIII, R'' = OH or CH_3) are deaminase inhibitors *in vitro*, as are the N^4-hydroxylamino analogues of cytidine, 2'-deoxycytidine, 5-methyl-2'-deoxycytidine and of 5-fluoro-2'-deoxycytidine. Although they are not deaminated by deaminases, they blocked rather than potentiated the effect of Ara-C in L1210 leukemic mice suggesting *in vivo* reduction of these hydroxylamino derivatives to deoxycitidines by reductases. Therefore, one structural requirement for potentially useful analogues (designed to block the deamination of Ara-C and thus potentiate its chemotherapeutic effect) is that they cannot be converted *in vivo* to deoxycytidines or to derivatives which act like deoxycytidines[34b]†.

Anhydronucleoside procedures were also used to prepare 5'-deoxy-Ara-C[32] and 1-α-L-xylofuranosylcytosine[35], the latter of which differs structurally from Ara-C only in the configuration at C-4'. Time does not permit a detailed discussion of their syntheses. Preliminary studies[27b] showed no activity, as expected, for these analogues in mouse leukemia, consistent with the generally accepted view that Ara-C exerts its chemotherapeutic effect on nucleotide levels. 5'-Deoxy-Ara-C was completely deaminated by human liver and mouse kidney homogenates whereas 1-α-L-xylofuranosylcytosine was not, indicating a high degree of specificity which the deaminase (s) possess for configurational alterations at C-4'. 5'-Deoxy-Ara-C was a weak deaminase inhibitor[27b].

To return to the question of alteration of the aglycon by use of anhydronucleosides, mention has been made that during our investigations into the synthesis of Ara-FU (see discussion for *Figures 5* and *21*) we noted varying instability of this nucleoside and its 2,2'-anhydro precursors in aqueous alkali. Similar instability is also exhibited by Ara-FC. Because of the interesting biological properties of these arabino nucleosides, we undertook a detailed investigation into the chemistry of these and related halogenated nucleosides in aqueous alkali[36, 37] (*Figure 22*).

When Ara-FU (LXVa or its 2,2'-anhydro precursor) is treated with 0·1N sodium hydroxide at ~ 60–70°, a rapid loss of selective absorption above 220 mμ occurs which is complete within 30 minutes. A *quantitative* yield of LXIXa was obtained which, unlike Ara-FU, was chemotherapeutically inert. Compound LXIXa did not consume metaperiodate, gave a positive ureido test, and showed negative aldehyde tests. The 5'-*O*-trityl derivative of Ara-FU also gave LXIXa. Ara-FC, when similarly treated with alkali, was also converted to LXIXa but in about 50 per cent yield. A clue to the structure of LXIXa was also provided by the fact that ribo-FU, 2'-deoxyribo-FU, and 2'-deoxylyxo-FU remained unchanged after 5 hour treatment with 0·1 N alkali at ~ 65°, whereas lyxo-FU did undergo loss of selective absorption in the ultraviolet under these conditions. These studies implicated the 2' "up" hydroxyl group in the saturation of the

† In a recent abstract, Hanze and Camiener report that 1-(β-D-ribofuranosyl)-4-hydroxytetrahydropyrimidin-2(1H)-one is a potent and specific inhibitor of the deamination of Ara-C by human liver homogenates. (See Abstr. 154th meeting, Amer. Chem. Soc., Chicago, September 1967).

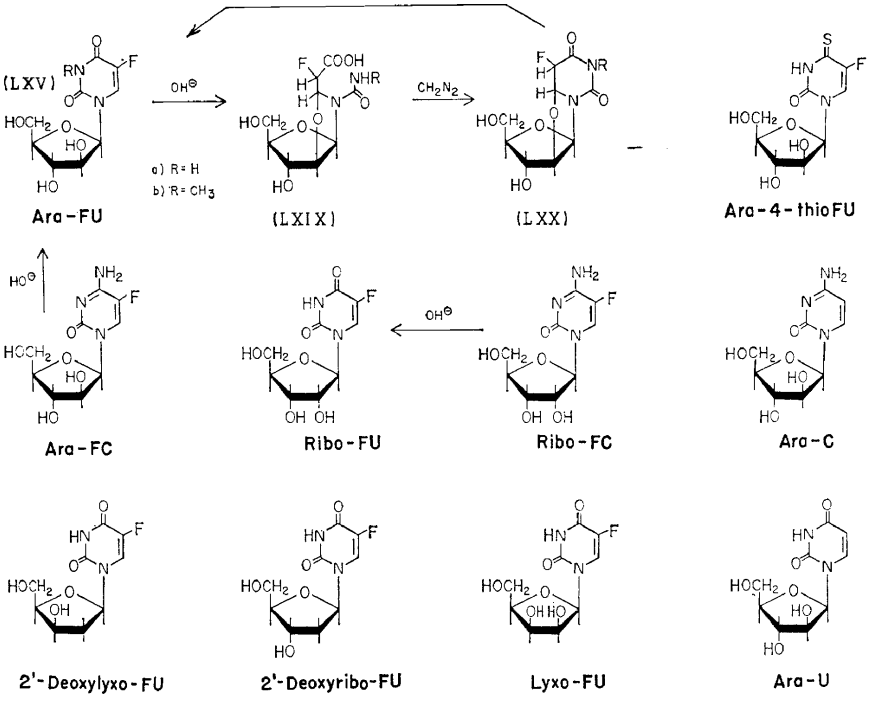


Figure 22. Reactions of Ara-FU, Ara-FC and related nucleosides in alkali. Synthesis of 6,2′-anhydronucleosides[36]

5,6-double bond of LXVa. N.m.r. data was also consistent with structure LXIXa as an open-chain ureide with a 6,2′-anhydro bridge.

Methylation of LXVa gave 3-methyl-Ara-FU (LXVb) which was very sensitive to alkali at room temperature. Under these conditions LXVb was rapidly converted to LXIXb. The n.m.r. spectrum of LXIXb was identical with that for LXIXa in DMSO-d_6 except for a doublet integrating for 3 protons at δ 2·64. This doublet (which becomes a singlet in dilute acid) proves the presence of an —$NHCH_3$ grouping which is rationalized only by the open-chain ureide structure LXIX.

The formation of LXIXa from Ara-FC may be due partially to its direct deamination to Ara-FU by alkali. We have observed that similar treatment of ribo-FC (5-fluorocytidine) with alkali produces some ribo-FU. Alternatively, Ara-FC may form the carboxamide analogue of the open-chain ureide (LXIXa), which, under the alkaline conditions employed, may have undergone partial hydrolysis to LXIXa. Incidentally, both Ara-U and Ara-C, upon alkaline treatment in the manner described, produce a much slower loss of selective absorption (which does not go to completion) suggesting that, with these unhalogenated arabino nucleosides, attack at C-6 by the 2′-hydroxy anion is also involved. It is noteworthy that Ara-4-thioFU remained unaltered by treatment with 0·1 N alkali under conditions which quantitatively converted Ara-FU to LXIX.

A plausible mechanism for the conversion of Ara-FU to LXIX is by the 1,4-addition mechanism as shown in *Figure 23*. The Ara-FU ion (**A**) (depicted in only one of several resonance structures which can be written for the aglycon) undergoes intramolecular nucleophilic attack by the 2′-hydroxyl

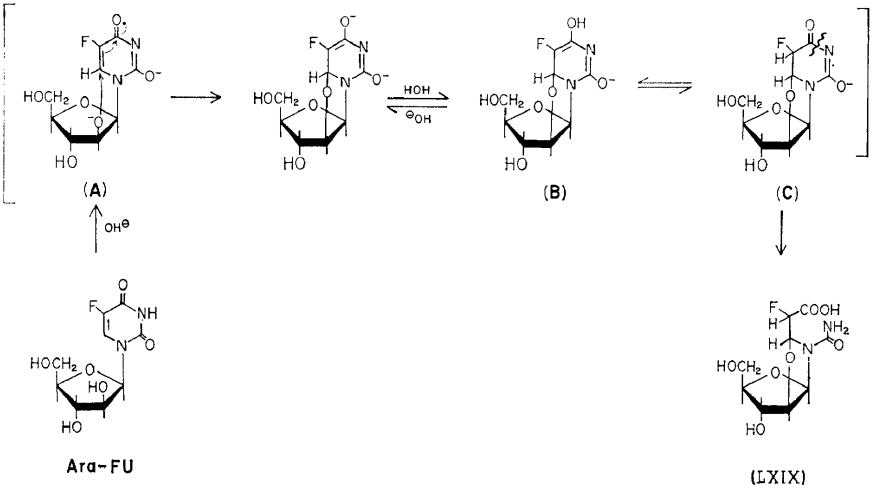


Figure 23. A mechanism for the conversion of Ara-FU to the 6,2′-anhydro open-chain ureide[36]

anion to give the 6,2′-anhydronucleoside (**B**) which tautomerizes to (**C**) and then undergoes ring cleavage to LXIX. The presence of a 5-fluoro atom should enhance the susceptibility of C-6 to nucleophilic attack. In support of this mechanism, Ara-4-thioFU is resistant to attack at C-6 in alkali. In the anion of Ara-4-thioFU, the negative charge would localize mainly on the sulphur atom which would mitigate against nucleophilic attack by the 2′-OH anion. The fact that 3-methyl-Ara-FU (LXVb) reacts readily to produce LXIXb is to be expected due to the absence of a formal negative charge in the aglycon.

Compounds LXIX are the first examples of the "6,2′-anhydro" structure in the nucleoside area. The formation of 6,5′-episulphide (cyclic) structures from certain 5′-thiol nucleoside derivatives had been reported[38a, b, c], however their formation was reversible leading to 5′-mercaptide anions or to disulphides with regeneration of the 5,6-double bond. Chang[38d] prepared a 6,5′-anhydro-5,5-diiodo-5,6-dihydrouridine by iodination of cytidine. This dihydro derivative splits out hydrogen iodide in alkali to regenerate the 5,6-double bond. Previously, Lipkin *et al.* reported (in abstract)[38e] the synthesis of a 6,5′-anhydrouridine unsaturated at the 5,6 positions by treatment of 5-iodouridine with base in dimethylsulphoxide. In all these cases[38] the anhydro structures contained a 7-membered ring fused to the 1 and 4 positions of the sugar moiety. In our case (*Figure 23*), double bond regeneration from intermediate (C) did not occur under our reaction conditions and 3,4-bond cleavage predominated, possibly as a result of strain introduced by the 1′-2′ fused 5-membered rings on the pyrimidine ring.

The ease with which the 2′-OH anion in the arabino ("up") configuration participates in the reaction of Ara-FC and Ara-FU is probably a general characteristic of pyrimidine nucleosides bearing the aglycon and the 2′-hydroxy in a *cis* relationship. Such a phenomenon might be responsible—at least in part—for the slow loss of ultraviolet absorption of Ara-C and Ara-U in warm aqueous alkali[36].

Regeneration of the double bond (LXIXa→ LXVb, *Figure 22*) was achieved however by treatment of LXIXa with diazomethane which gave two neutral products one of which was LXXb. The major component in this reaction was LXVb. It is clear that esterification of LXIX enhances ring closure to LXX which, under these reaction conditions, undergoes elimination of the alcoholic group from C_5–C_6 of LXX[36].

Further examination of the properties of the 6,2′-anhydro-5-fluoro-5,6-dihydro nucleoside LXIX led to the discovery[37a] of a new rearrangement of the aglycon of 5-halogenated arabinosyl nucleosides (*Figure 24*). Reaction of LXIX with N sodium hydroxide for 20 hours at 60° yielded a crystalline product (LXXI, 54%) which was fluorine-free and analyzed for $C_9H_{12}N_2O_4$. The same product (LXXI) was also obtained directly from Ara-BrU (LXXIIa) after 3 hours under similar reaction conditions in 61 per cent yield. The following data shows that LXXI is 1-(β-D-arabinofuranosyl)-2-oxo-4-imidazoline-4-carboxylic acid and that the overall formation of LXXI from LXV or LXXIIa involved a ring contraction hitherto unreported in the nucleoside area.

Compound LXXI formed a methyl ester (LXXIIIa) with one mole of diazomethane, gave a crystalline tri-*O*-acetate (LXXIIIb), and consumed one mole of metaperiodate per mole *slowly* consistent with an α-*trans*-glycol system. The n.m.r. spectrum of LXXI in DMSO–d_6 was consistent with the structure depicted showing a single NH proton at δ 10·65 which was long-range coupled (J = 1·5) with a single vinylic proton at 7·32. (Both the long-range coupling and the NH signal disappeared upon addition of D_2O). When the 3-methyl derivative of Ara-BrU (LXXIIb) was treated with alkali, then methylated with excess diazomethane, and finally acetylated with acetic anhydride, crystalline LXXIIIc was obtained which was identical with the product afforded by alkylation and acetylation of LXXI. The *N*-methyl signal of LXXIIIc was a singlet thus establishing the cyclic nature of the aglycon. The ultraviolet absorption properties of LXXI and its derivatives were generally similar (at pH 1 and 7) to alkylated 2-oxo-4-imidazoline-4-carboxylic acids.

Attempts to degrade LXXI to the free aglycon under a variety of acidic conditions were unsuccessful. However, oxidation of ester LXXIIIa with metaperiodate followed by treatment with phenylhydrazine gave the crystalline bisphenylhydrazone LXXIV. Condensation of the imidazoline methyl ester LXXV with tetra-*O*-acetyl-α-D-glucosyl bromide gave a mixture of the *N*-1 and *N*3-glucosylated nucleosides. The major product isolated (LXXVI) was deacetylated, oxidized with metaperiodate, and then treated with phenylhydrazine to give the bisphenylhydrazone LXXIV which had been obtained previously from LXXI.

A plausible mechanism (*Figure 24*) for the conversion of LXIX (or LXXII) to the imidazolone nucleoside would involve first a nucleophilic attack of

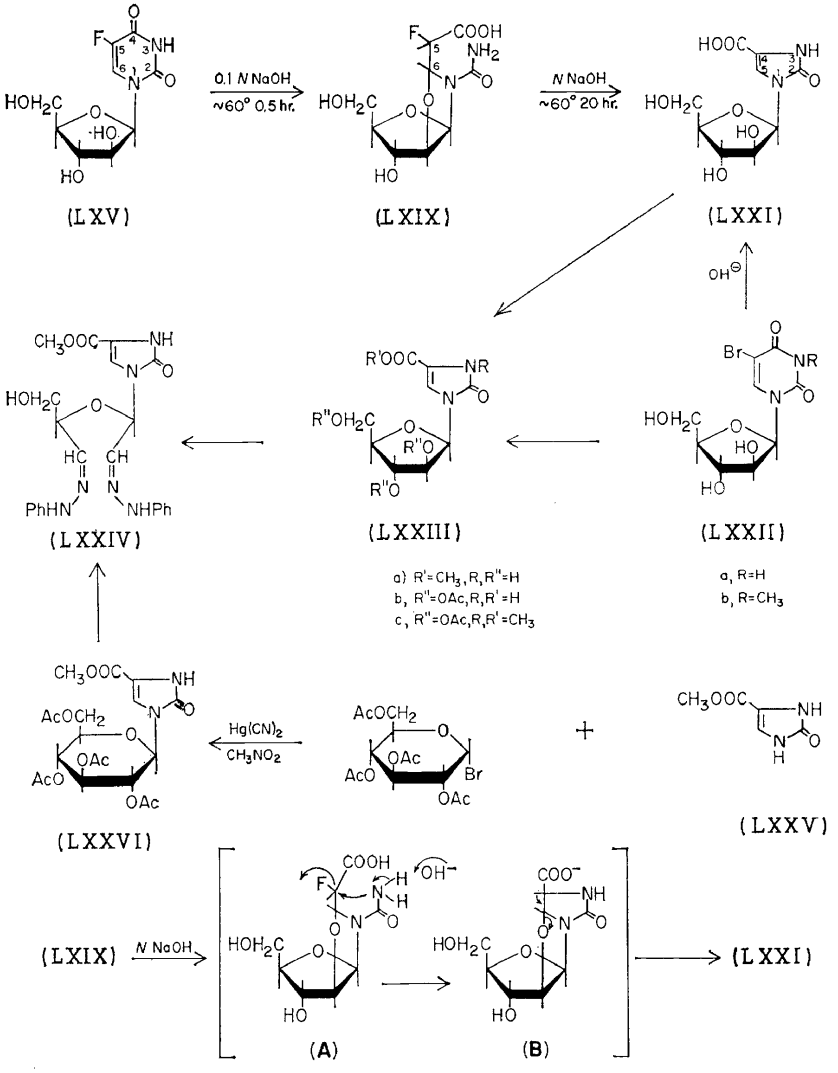


Figure 24. Syntheses and proof of structure of 1-β-D-arabinofuranosyl-2-oxo-4-imidazoline-4-carboxylic acid[37a]

the amide nitrogen of intermediate [**A**] on C-5 with the displacement of the halogen atom (fluorine or bromine) to give intermediate [**B**]. Elimination of the sugar alcohol from [**B**] (loss of the 6,2′-anhydro linkage) would produce LXXI[37a].

We are currently engaged in an intensive study[37b] of certain 5-halogenated pyrimidine *ribo*nucleosides and our preliminary results are rather encouraging. It will be recalled that 5-fluorouridine (*Figure 22*) did not react with 0·1 N (and 1 N) alkali under conditions which rapidly converted Ara-FU to the 6,2′-anhydro open chain ureide LXIX[36]. Reist

et al.[38c], observed that at pH 1–7 a solution of 5′-thiouridine exists ~ 20 per cent in the isomeric 6,5′-cyclic sulphide structure, whereas Chambers and Kurkov[38b] showed that 2′,3′-*O*-isopropylidene-5′-thiouridine was cyclized completely to the 6,5′-anhydronucleoside at pH 3–7. They concluded that the isopropylidene group "forces the sugar ring into a conformation that favours the proximity of the thiol group to the uracil double bond."[38c] Such considerations are also indicated by the different sets of coupling constants for H1′-H2′ 4′ exhibited by 5-fluorouridine and its 2′,3′-*O*-isopropylidene derivative[37b]. We therefore investigated the reactivities of 2′,3′-*O*-isopropylidene-5-halogenated uridines in aqueous alkali (*Figure 25*).

Treatment of isopropylidene-5-fluorouridine (LXXVIIa) with N alkali at 55° produced a rapid partial loss of ultraviolet absorption at 268 mμ and the slower appearance of a new maximum at 252 mμ (measured at pH 7·5). After treatment of the reaction mixture with Dowex 50 (H^+) a crystalline material LXXVIIIa (~ 60%) was obtained with ultraviolet and n.m.r. spectral characteristics similar to the imidazolone nucleoside (LXXI of *Figure 24*). When the 5-bromo analogue LXXVIIb was similarly treated with alkali, the ultraviolet spectral pattern of the monitored reaction was different. The loss of the absorption band at 275 mμ occurred concomitantly with the appearance of a new band at ~ 260 mμ. Electrophoretic examination of this reaction mixture indicated a second product, along with LXXVIII. When the 5-iodo analogue (LXXVIIc) was treated with N alkali at 55°, only a trace of imidazolone nucleoside formed. The major product isolated in crystalline form (~ 30%) was the 6,5′-anhydro-2,3-*O*-isopropylidene derivative (LXXIX). The ultraviolet spectrum of LXXIX was similar to that exhibited by a sample of the 2′,3′-unsubstituted-6,5′-anhydro-ribosylbarbituric acid kindly provided by Lipkin. [An abstract dealing with the preparation of this sample by "alkaline hydrolysis of 5-iodouridine" had appeared[38e]]. The n.m.r. spectrum in DMSO-d_6 of LXXIX exhibited a single vinylic proton for H-5 and a widely spaced quartet (centred at δ 4·4, J = ~ 13 C/S for the gem hydrogens at C5′) which we have found to be characteristic[39] of anhydronucleosides containing an oxygen bridge from the aglycon to C5′.

When 5-bromo- and 5-iodo-uridine were treated with alkali under the same conditions employed for their isopropylidenated derivatives, the reaction was much slower. Ultraviolet data indicated that possibly some 6,5′-anhydro-ribosylbarbituric acid had formed, as was noted by Lipkin[38e] in the hydrolysis of 5-iodouridine.

A possible explanation for the results observed for the alkaline treatment of the isopropylidene-5-halogenouridines (LXXVII) is shown in *Figure 25*[37b]. With the fluoro analogue (LXXVIIa) the initial loss of ultraviolet extinction at 268 mμ is best explained as a result of nucleophilic attack by the 5′-hydroxyl anion on C-6 with the formation of [**C**] via intermediates [**A**] and [**B**]. The conversion of [**C**] to imidazolone LXXVIII proceeds by nucleophilic displacement of the 5-fluoro atom by the amide nitrogen followed by elimination of the sugar alcohol as previously described for the *arabino* analogue. With the 5-bromo analogue (LXXVIIb) elimination of hydrogen halide from intermediate [**B**] is more favoured over the ring cleavage so that both LXXIX and LXXVIII are formed. With the iodo

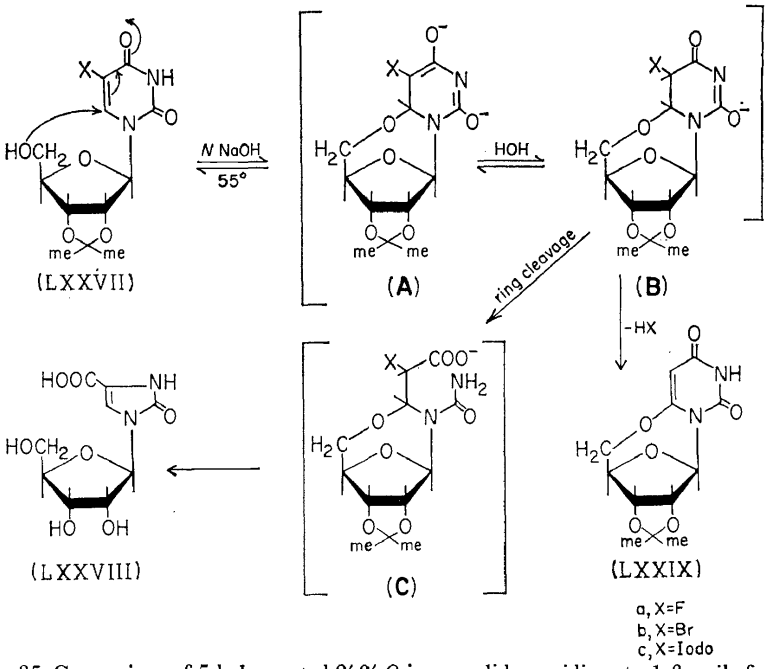


Figure 25. Conversions of 5-halogenated-2′,3′-*O*-isopropylideneuridines to 1-β-D-ribofuranosyl-2-oxo-4-imidazoline-4-carboxylic acid and/or the acetonide of 6,5′-anhydro-1-(β-D-ribofuranosyl)-barbituric acid.[37a,b]

analogue (LXXVIIc), the elimination of hydrogen iodide from [**B**] predominates so that LXXIX is the major product and only a trace of LXXVIII is in evidence.

In all of the alkaline reactions of halogenated nucleosides described in *Figures 25* and *24*, some ammonia was evolved which probably accounts to a major degree for the relatively low yields (40–60%) of imidazolone nucleosides or of the 6,5′-anhydro derivative LXXIX. It should also be noted that with alkaline treatment of LXXVIIb and LXXVIIc, some 5-hydroxyuridine formation is indicated by the appearance of a small ultraviolet maximum at ~ 307 mμ.*

Another consideration in these reactions is the possible formation of barbituric acid nucleosides by direct attack by hydroxide ion, for example, on C-6 of LXXVII. In this regard, our current investigations of 5-halogenated-1-methyluracils are relevant (*Figure 26*)[37a]. Treatment of 1-methyl-5-bromouracil (LXXX) with N alkali at ~ 60° gave a fairly rapid conversion to 1-methylbarbituric acid (LXXXII) which was isolated in ~ 50 per cent yield in crystalline form. [Fikus *et al.*[40], had reported the *photochemical* conversion of a pH ~ 6 solution of 1,3-dimethyl-5-fluorouracil to photoproducts, one of which was identified as 1,3-dimethylbarbituric acid]. 1-Methylbarbituric acid, however, undergoes a slower decomposition under these alkaline conditions to product(s) which at pH ~ 7–8 are devoid

* *Added in Proof:* Further studies [37b] have shown that the isopropylidene-5-hydroxyuridine formed is stable in N alkali. In weaker alkali, it is converted almost quantitatively into the imidazolone nucleoside LXXVIII by a different mechanism.

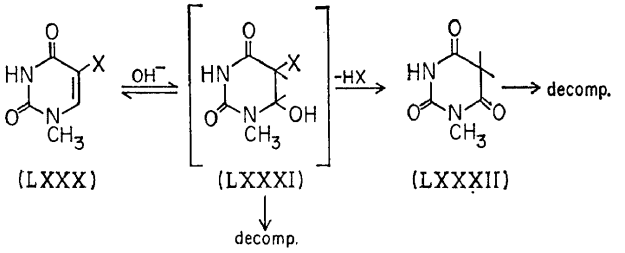


Figure 26. Conversion of 1-methyl-5-bromouracil in alkali to 1-methylbarbituric acid.[37a,b]

of selective absorption in the ultraviolet. This reaction of LXXX (X = Br) in warm alkai may be monitored spectrally by periodic removal of aliquots which are diluted and read at pH ~ 7·5. One observes the disappearance of the maximum at 278 mμ with the concomitant appearance of a maximum of much higher extinction at ~ 258 mμ, a trend which is almost complete in ~ 6 hours. Over a 24 hour period, selective absorption is then lost. The formation of 1-methylbarbituric acid (LXXXII) from LXXX (X = Br) is readily explained by the equilibrium LXXX ⇋ LXXXI (attack of hydroxide ion on C-6). Elimination of the elements of hydrogen bromide from LXXXI would lead to the irreversible formation of 1-methylbarbituric acid.

1-Methyl-5-fluorouracil (LXXX, X = F), on the other hand, is relatively stable* to these alkaline conditions. Over a 24 hour period in warm, N alkali, only a 10–15 per cent loss in extinction at 271 mμ is observed as determined from aliquots measured at pH 7–8. This small loss in extinction is due probably to the formation and subsequent degradation of intermediate LXXXI (X = F) to fluoromalonaldehydic acid[37b]. Support for the slow formation of some fluoromalonaldehydic acid from LXXXI derives by analogy with the studies of Lozeron *et al.*[41] who had noted that 5,6-dihydro-5-fluoro-6-hydroxyuracil is converted in alkali to urea and fluoromalonaldehydic acid. Conceivably, this latter type of degradation could also occur with the 5-bromo analogue. Apparently, however, elimination of HBr from LXXXI (X = Br) predominates and methylbarbituric acid forms[37b]. The importance of these studies is that if any barbituric acid nucleoside formed during the treatment of 5-bromouridine or its acetonide (LXXVIIb, *Figure 25*) with warm alkali, such barbiturates would probably be decomposed with time under the reaction conditions employed.

Since the 5-fluorouridine derivative LXXVIIa is less readily accessible than its bromo analogue LXXVIIb, experiments were conducted with the latter analogue in order to develop a more practical approach to the riboimidazolone nucleoside (LXXVIII). Treatment of LXXVIIb with 0·1 N alkali at reflux temperature afforded ~ 50 per cent yield of LXXVIII as the major product isolated.

* The term "stable" refers only to degradative reactions. During these studies it was found[37c] that 1-methyl-5-fluorouracil, 5-fluorouridine, FUDR, and 5-fluorouracil are easily converted in warm sodium deuteroxide to their 6-deutero analogues by a base catalyzed exchange. This method should be of value in the preparation of selectively-labelled derivatives of these biologically-important compounds.

The 2-oxo-4-imidazoline nucleosides offer many possibilities as starting materials for the syntheses of compounds of potential biochemical interest. For example, the structural resemblance between the carboxamide analogue of LXXVIII and 5-amino-4-imidazole carboxamide ribonucleoside ("AICAR") opens many facets for synthetic studies with this class of nucleosides. We are encouraged by the fact that preliminary studies[27b] at our Institute have shown that the riboimidazolone (LXXVIII) is active against Burkitt's tumor cell cultures at concentrations of $\sim$ 1–3 γ/ml.

All the anhydronucleosides discussed thus far contain an oxygen bridge between the aglycone and the sugar moiety. We will now consider the introduction of nitrogen into anhydronucleosides as the bridging heteroatom (*Figure 27*)[39].

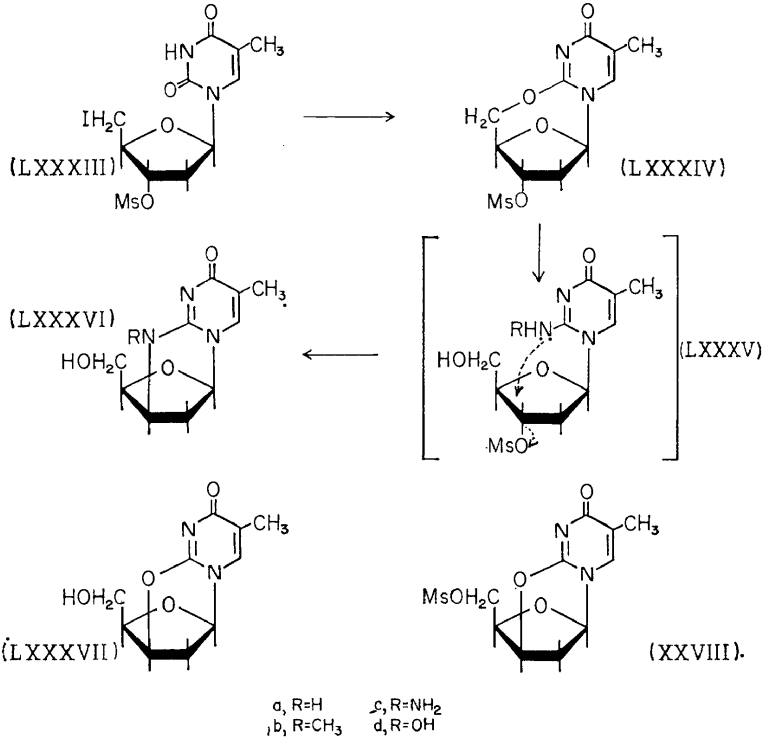


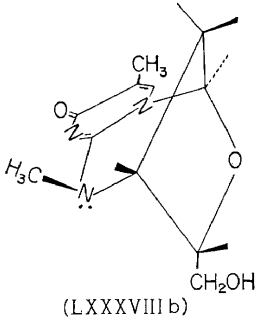
Figure 27. Syntheses of 2,3′-imino-bridged "anhydro" nucleosides[39]

The approach requires an anhydronucleoside intermediate containing a leaving group in the sugar moiety. As a model compound, we selected 2,5′-anhydro-3′-*O*-mesylthymidine (LXXXIV) which was prepared by reaction of the known[15] iodonucleoside LXXXIII with silver acetate in

methanol. Treatment of LXXXIV with liquid ammonia for 5 days at room temperature gave a high yield of a crystalline product which analyzed for LXXXVIa and exhibited ultraviolet spectral characteristics similar to isocytosine nucleosides. The n.m.r. spectrum of LXXXVIa in DMSO-d_6 showed as expected, the H-3′ signal as a broad multiplet centred at δ 3·52 which is shifted considerably upfield when compared to the H-3′ signal ($\delta = 5{\cdot}31$) of the "isosteric" anhydronucleoside LXXXVII. Final confirmation of the 2,3′-imino structure of LXXXVIa was obtained by the synthesis of LXXXVIb by reaction of LXXXIV with methylamine. The n.m.r. spectrum of LXXXVIb exhibited (in addition to the C-5 methyl doublet at δ 1·76) a sharp *singlet* for the N—CH_3 grouping which establishes the 2,3′-imino bridge in LXXXVI. It is also clear, that in the conversion of LXXXIV→LXXXVI by amines, the isocytidine type derivatives (LXXXV) were intermediates. Treatment of LXXXIV with anhydrous hydrazine or with methanolic hydroxylamine gave the corresponding derivatives, LXXXVIc and LXXXVId.

The hydrazino analogue (LXXXVIc) forms a hydrazone derivative with benzaldehyde. With nitrous acid, the exocyclic amino group (R) of LXXXVIc is lost and LXXXVIa is obtained.

A comparison of the properties of the 2,3′-imino bridge nucleoside (LXXXVIa) with the "oxygen isostere" (LXXXVII) is of interest. The 2,3′ -imino nucleoside is far more stable to acid or alkali than is (LXXXVII)[39]. Whereas LXXXVII is easily cleaved in base, LXXXVIa is stable to refluxing N sodium hydroxide as well as to alkoxide. Treatment of the 2,3′-imino nucleoside (LXXXVIa) with N hydrochloric acid at reflux temperature for one hour produced only a small amount of degradation; though, after 2 days at reflux temperature, glycosyl cleavage was extensive. This slow degradation of LXXXVIa[39] is to be contrasted with the report[15] of the relatively rapid glycosyl cleavage of the 2,3′-anhydro isostere (LXXXVII) within one hour under these reaction conditions.



(LXXXVIIIb)

The 2,3′-iminonucleosides (LXXXVI) may be viewed as derivatives of 2,4-diaza-6-oxabicyclo(3.2.1)octane shown conformationally in structure (LXXXVIIIb). The *N*-methyl group in LXXXVIIIb probably exists mainly as the *exo*-conformer. The alternate *endo*-conformer would not be favoured due to steric hindrance imposed by the bulky 4′-hydroxymethyl group of the sugar moiety[39].

The first synthesis of an anhydronucleoside containing sulphur as the bridging heteroatom was achieved (*Figure 28*) by Shaw and Warrener[42] who showed that mesylation of 5′-*O*-trityl-2-thio-5-methyluridine (LXXXIX) gave directly a high yield of the S2,2′-anhydronucleoside

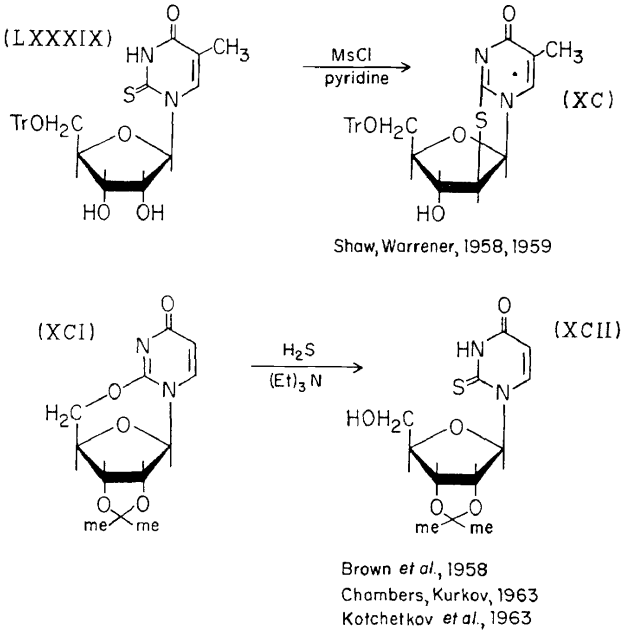


Figure 28

(XC). Their method was preceded by a total synthesis of 2-thio-5-methyluridine. Brown and coworkers[24] demonstrated that reaction of 2,5′-anhydroisopropylideneuridine (XCI) with hydrogen sulphide in DMF in the presence of triethylamine gave several products, one of which was the acetonide of 2-thiouridine XCII. Other researchers[38b, 43] have investigated aspects of this latter reaction.

Our current excursion into the synthesis of S-bridged anhydronucleosides[44] is an adaptation of the approach[39] we used for the synthesis of the 2,3′-imino bridge nucleosides. When the 2,5′-anhydronucleoside LXXXIV (*Figure 29*) was heated with methanol containing triethylamine, a good yield of the 2-methoxy nucleoside XCIII was obtained. Treatment of XCIII with hydrogen sulphide and triethylamine in DMF at 65° for several hours afforded a simple route to the S2,3′-anhydronucleoside XCIV. Cleavage of the sulphide bridge of XCIV with hot 0·1 N aqueous alkali gave a fair yield of the 3′-mercapto nucleoside XCV. It is obvious that the 2-thionucleoside XCVI, was an intermediate in the conversion of XCIII → XCIV. Indeed, nucleoside XCVI was synthesized by treatment of LXXXIV at room temperature with hydrogen sulphide in DMF and triethylamine. Compound XCVI was converted to XCIV simply by boiling

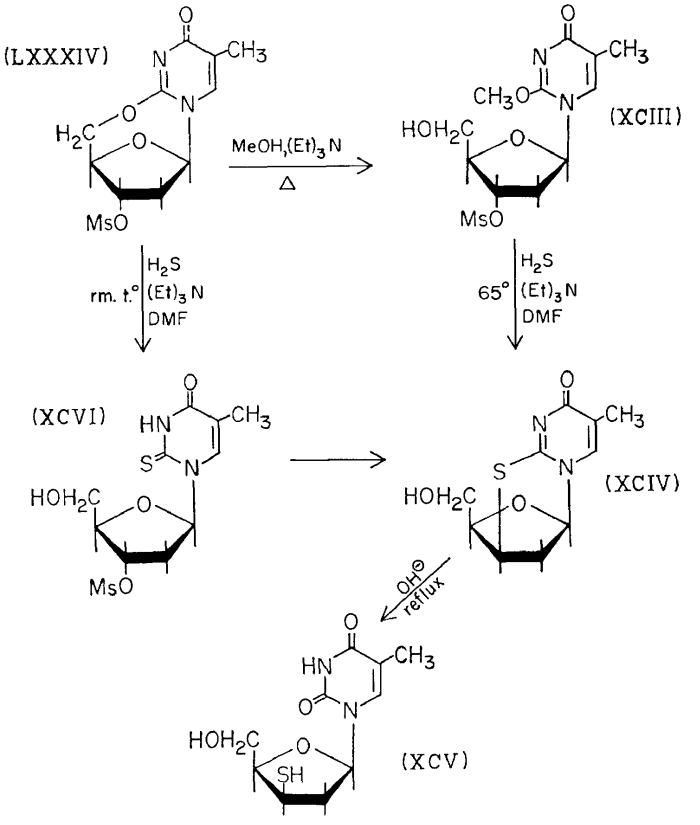


Figure 29. Synthesis of S2,3′-anhydronucleosides.[44]

it in ethanol containing some triethylamine. Further studies in this area are underway in our laboratories.

In conclusion, it is clear that anhydronucleosides will continue to play an ever increasing role in the opening of new facets in the chemistry of nucleosides and in the syntheses of new agents of biological interest.

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