New syntheses of 1,3 and 5 substituted uracils, 1,4 and 5 substituted pyrazoles, and 1,4 and 6 substituted pyrazolo [3,4-d]pyrimidines, including glycosides related to naturally occurring pyrimidines, imidazoles, purines and their nucleoside derivatives.

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by

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March 1978.
To my family and the memory of my father.
PREFACE

Some compounds, analogous to those found in naturally occurring systems, are found to possess chemotherapeutic activity. Some, in the form of their nucleoside or nucleotide derivatives, are valuable antimetabolites in that they may block normal RNA or DNA polymerisation, or may be incorporated into nucleic acids to form fraudulent, but not necessarily defective, polymers. Modification of natural ring systems, with a view to promoting chemotherapeutic activity is therefore of considerable interest; variation in the position and nature of the modification or ring substituent having a marked effect on chemotherapeutic activity.

It is the purpose of this thesis to suggest methods for the facile synthesis of various uracils, pyrazoles and pyrazolo[3,4-d]-pyrimidines with alkyl, aryl and glycosyl substituents such that the nature of the ring substituents is easily varied.

To this end a number of ethoxymethylene reagents were prepared which, by reaction with primary amines and hydrazines, would give acyclic intermediates capable of easy cyclisation into the uracil, pyrazole and pyrazolo[3,4-d]pyrimidine ring systems. Variation in the nature of specific substituents being determined by the choice of amine or hydrazine, other substituents being varied by modification of the original reagent.

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B.G.H.
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routes to, some pyrimidines, pyrazoles, purines and
pyrazolo[3,4-d]pyrimidines, and their nucleosides.
a) Introduction

It was known early in the history of organic chemistry that compounds containing the pyrimidine ring system were produced as breakdown products of uric acid.

In 1818 Gaspare Brugnatelli isolated alloxan by the oxidation of uric acid with nitric acid, and in 1848 Frankland and Kolbe carried out the first primary synthesis of a pyrimidine by the action of metallic sodium on propionitrile, when 4-amino-2,6-diethyl-5-methylpyrimidine was obtained. The next important advance perhaps was the synthesis of barbituric acid from malonic acid and urea by Grimaux in 1878 and was the first example of what has since become known, by some, as the Principal Synthetic Method. This method involves the fusion of a three carbon fragment containing such functional groups as, for example, aldehyde, keto, ester or cyano groups on carbon atoms 1 and 3, with a one carbon two nitrogen fragment, for example, urea, guanidine or a substituted amidine.

It was not until 1885, however, that Pinner, having made his first pyrimidine derivative in the previous year, postulated that pyrimidine was in fact a hexagonal counterpart of benzene, pyridine and triazine. More recent X-ray and dipole moment studies have supported this view and the planar ring form (1) is now accepted.

A more systematic name for the 1,3-dinitrogen hexagonal heterocycle was suggested by Widmann in 1888 namely, diazine or miazine, but the trivial name had by then become too well
Pyrimidine can, like its 5 member ring analogue imidazole, be regarded as a cyclic amidine and this strongly dictates the behaviour of its derivatives. Thus whereas positions 2, 4 and 6 are of very similar character, the 5 position is characterised by its much more aromatic nature. The equivalence of positions 2, 4 and 6 can render unambiguous numbering difficult. However as the pyrimidine ring most often mentioned in this thesis will be that of uracil, the ring will in most cases be drawn and in all cases numbered as shown.

Uracil is thus named 2,4-dihydroxypyrimidine and not 2,6-dihydroxypyrimidine. This system is accepted by Chemical Abstracts, Ring Index and the Chemical Society, although the latter journal also accepts the more detailed name 1,2,3,4-tetrahydro-2,4-dioxopyrimidine, however for ease of writing, the simpler nomenclature will be used throughout this thesis.

One further difficulty concerning the nomenclature of substituted pyrimidines arises when the ring carries one or more potentially tautomeric substituents, in the 2, 4 or 6 positions.

Thus (3) could be named 4-hydroxypyrimidine; 4-pyrimidol; 4-pyrimidone or 1,4-dihydro-4-oxo-pyrimidine. However, in this thesis all such compounds are named as hydroxy, mercapto or amino derivatives, whatever the evidence for the predominant tautomeric form may indicate, or by accepted trivial names, e.g. 2-thiouracil(4).
The pyrimidine ring is to be found in many important naturally occurring compounds. Thiamine, or vitamin B₁ (5a) is essential as a component in the human diet; in its absence, the human vitamin deficiency known as beriberi develops. Fortunately, rich resources of thiamine are available in eggs and yeast and the vitamin occurs widely in plants.

In the form of its pyrophosphate (5b) (also known as cocarboxylase), the substance participates in several biological reactions, among which may be cited, the enzymic conversion of pyruvate to acetylcoenzyme-A, and to acetoin, the enzymic decarboxylation of α-ketoglutarate and the transformations catalysed by yeast carboxylase and transketolase.

Nucleic acids have long been known to contain the pyrimidine bases, uracil (2), thymine (6), cytosine (7), and in addition 5-methylcytosine (8) and 5-hydroxymethylcytosine (9) also are present in some nucleic acids.

In 1958, in addition to adenine (10) and guanine (11), four other purines were isolated from several nucleic acid sources. The other purines were, namely, 2-methyladenine (12), 6-methylaminopurine (13), 2-methylaminoguanine (14) and 2-dimethylaminoguanine (15).

Both types of nucleic acids (RNA and DNA) contain adenine (10) and guanine (11). Cytosine (7) also occurs in both types of nucleic acid, but uracil (2) occurs only in RNA. 5-Methylcytosine (8) has been found to be a fairly common constituent of DNA but only occurs in minute quantities in RNA. 5-Hydroxycytosine (9) has been found in certain DNA's.
(5)

(2)  (6)  (7)  (8)  (9)

(10)  (11)  (12)

(13)  (14)  (15)

(16)

a) $R = H$

b) $R = \text{P-O-P-OH}$
The substitution of the sugar moiety (ribofuranoside or deoxyribofuranoside) at position 1 in the pyrimidine ring and position 9 in the purine ring, with $\beta$-configuration of the furanose link gives rise to a nucleoside, e.g. 1-$\beta$-D-ribofuranosylcytosine (16a). Phosphorylation of (16a) at the 3' position gives a nucleotide (16b). Nucleic acids are polyphosphodiesters formed by the joining of the 3'phosphate of one nucleotide with the 5' hydroxyl of the next.

**Analogues of naturally occurring Pyrimidines**

Structural analogues of the pyrimidines are of considerable interest as potential chemotherapeutic reagents (e.g. antimetabolites). Certain p-aminobenzenesulphonyl derivatives of aminopyrimidines have valuable bacteriostatic properties in vivo. Of the many sulphonamides tested, 2-p-aminobenzenesulphonamido-pyrimidine and its 4-methyl and 4,6-dimethyl derivatives are particularly important and are known as sulphadiazine (17), sulphamerazine (18) and sulphamethazine (19) respectively. The bacteriocidal activity is lost if any further substituent is present in the benzene ring.

Sulphamerazine is important since it is rapidly absorbed and slowly excreted, thereby maintaining a higher and more persistent blood level than other sulphonamides at the same dosage. It is less likely to cause urinary obstruction than sulphadiazine, being more soluble, and its therapeutic activity in respiratory diseases appears to be similar.
2-Sulphanilamido-5-chloropyrimidine(20) and 2-metanilamido-5-chloropyrimidine (metachloridine) are unusual and effective antimalarial sulphonamides. Many hydrazino compounds including 4-hydrazinopyrimidine have been examined for antibacterial activity, and many mono- and dimethylpyrimidine derivatives bearing an amino- and a dialkylamino- or alkylamino substituent have been synthesised and a number have marked antimalarial activity.

Several dihydro-4,6-dihydroxypyrimidines or the corresponding 2-alkoxy- compounds have been prepared by the hydrogenolysis of a variety of 5,5-disubstituted 2-thiobarbituric acids. In particular, 4,6-dihydroxy-5-phenyldihydropyrimidine (mysoline primidone) is obtained by reduction of 5-ethyl-5-phenyl-2-thiobarbituric acid, and is found to be an effective anticonvulsant.

Barbituric acid derivatives, barbiturates with two substituents in the 5-position, have been extensively investigated because of their various chemotherapeutic properties. They are important depressants for the central nervous system, the effect varying from light sedation to general anaesthesia, depending on the choice of drug, dose and method of administration.

Barbitone (veronal) (22), 5,5-diethylbarbituric acid, the first barbiturate to be used therapeutically, and its sodium salt, sodium barbitone (medinal) were superseded by phenobarbitone (luminal) (23), 5-ethyl-5-phenylbarbituric acid. Luminal is widely used as a sedative for the treatment of epilepsy, although phemitone (prominal) (24), 1-methylphenobarbitone, is said to be more effective for this purpose.
$$R = \text{H}_2\text{N-SO}_3\text{H}$$

\[
\begin{align*}
&\text{\textbf{(17)}} \\
&\text{\textbf{(18)}} \\
&\text{\textbf{(19)}} \\
&\text{\textbf{(20)}} \\
&\text{\textbf{(21)}} \\
&\text{\textbf{(22)}} \\
&\text{\textbf{(23)}} \\
&\text{\textbf{(24)}} 
\end{align*}
\]
Of intermediate duration are the drugs butobarbitone (soneryl) the 5-ethyl-5-n-butyl- compound, pentobarbitone (nembutal), the 5-α-methylbutyl- compound and amylobarbitone (amytal), the 5-isoamyl- compound.

Short acting barbiturates include cyclobarbitone (phanodorn), 5-ethyl-5-(Δ3 cyclohexenyl)barbituric acid, and seconal, the 5-allyl-5-α-methylbutyl- compound. Some barbiturates are rapidly removed in the body and these include hexobarbitone (evipan), sodium-5-(Δ3 cyclohexenyl)-1,5-dimethylbarbituric acid, which is now however replaced by thiopentone (pentathol), sodium-5-ethyl-5-α-methylbutyl-2-thiobarbiturate, as the standard drug for intravenous induction of general anaesthesia.

Of the seven possible isomers of monoaminodihydroxypyrimidines, some are useful as growth inhibitors, e.g. on streptococcus faecalis. 5-Aminouracil inhibits lactobacillus casei and 6-aminouracil inhibits the growth of the Crocker sarcoma significantly.

A diaminohydroxypyrimidine, Vicine, 2,4-diamino-6-hydroxy-pyrimidine-5-β-D-glucopyranoside, is considered to be responsible for the toxicity of Lathyrus ciciro (vetch) to chickens.
b) Established synthetic routes to the pyrimidine ring system.

Four main routes have been detailed in the literature for the synthesis of the pyrimidine skeleton. Three are represented schematically below, the fourth involves cyclisation of a preformed linear intermediate.

\[
\begin{align*}
\text{N} & \quad \text{N} \\
\text{C} & \quad \text{C} \\
\text{C} & \quad \text{N} \\
\end{align*}
\]

a) The Principal Synthetic Method

One of the most useful synthetic routes is that in which a three carbon fragment is condensed with urea or an amidine. The order of reactivity of the latter compounds is guanidine > thiourea > alkylthioureas > amidines > urea. One result of this variation in reactivity, is that 2-hydroxy compounds are often prepared via the 2-mercapto derivatives, since urea sometimes does not react satisfactorily.

The condensations are usually base-catalysed, and most often carried out by refluxing with sodium ethoxide in ethanol, as in the synthesis of barbituric acid(25), from diethylmalonate and urea. Various barbiturates, e.g. 5,5-diethylbarbituric acid (veronal), and 5-ethyl-5-phenylbarbituric acid (phenobarbitone), are obtained by the use of the appropriate malonic esters.

Guanidine(26) condenses with ethyl cyanoacetate(27) to give 2,6-diamino-4-hydroxypyrimidine(28), an important intermediate in the synthesis of pteridines as well as purines.

Thymine(6) may be obtained by condensing thiourea with the
formylacetic ester(29) and subsequent hydrolysis, but a better yield is obtained from urea and the cyanoacetic acid(30), which in the presence of acetic anhydride, gives methyl cyanoacetyl urea(31). (31) is reduced to thymine(6) and ammonia with hydrogen and platinic oxide.

Uracil(2) is best obtained by heating a solution of malic acid(32) and urea in fuming sulphuric acid.

An elegant synthesis makes cytosine more readily available. Condensation of malondialdehyde diethylacetal(33) with hydroxylamine hydrochloride gives isoxazole(34), which is converted to β-ethoxyacrylonitrile(35) by treatment with diethylsulphate and is cyclised to the required product in butanol using sodium butoxide.

Some substituted cytosines can be made by the reaction of urea or thiourea with ethyl cyanoacetate and triethyl orthoformate in the presence of acetic anhydride. The intermediate(36) is cyclised to yield the 2-hydroxy(mercapto)-4-aminopyrimidine(37). However it will be noted that in the cyclisation of (36), an alternative reaction is possible, namely, condensation between the ethoxycarbonyl group and the free primary amine liberating ethanol and yielding a 5-cyano-4-hydroxy compound(38). This is the main reaction when X= -SEt.

The pyrimidine(42), required for the synthesis of thiamine(5) (vitamin B₁), has been prepared by many methods, one of which is outlined below. Acetamidine(39) was condensed with ethoxy-methylene malonic ester(40) to give the basic ring structure (41) and the required substituents were obtained as shown in fig. 1.
2-Methyl-4-amino-5-bromoethylpyrimidine hydrobromide(42) was then coupled with (43) to give thiamine(5).

b) **Route two**

The second route to the pyrimidine skeleton involves the fusion of a three carbon, one nitrogen fragment, with a one carbon, one nitrogen fragment, e.g. an amidine. The reaction of formamidine(44) with malononitrile(45), might be expected to yield 4,6-diaminopyrimidine(46), but two molecules of the amidine are involved. The first reacts with the active methylene group of malononitrile to give the acyclic compound (47), and the second condenses with this, to give 4-amino-5-cyano.pyrimidine(48).

Perhaps the most common syntheses of this type can be summarised in the reaction scheme shown in fig. 2, in which the eliminated group A of the imine(50), may be -OEt; -OH; -SH or -NH₂. The required aminomethylene intermediate(49) may be obtained from the corresponding ethoxymethylene compound(51) and ammonia, but it can also result from the reaction of an imino ether, amide, or as just described, an amidine with the reactive methylene compound(52), as a preliminary to cyclisation with the same reagent. The success of the cyclisation depends on the nucleophilic character of the aminomethylene group, or its derived anion, which will increase if X is -CN, rather than -COOEt. Therefore, malononitrile would give good yields of the pyrimidine by this type of synthesis with all amidines, whereas ethyl cyanoacetate tends to stop at the formation of the aminomethylene intermediate.
In a similar series of ring syntheses, an aminomethylene compound (53) reacted with an isocyanate, yielding a ureide (54), which cyclised in a succeeding stage e.g. to yield the pyrimidine (55).

The earliest recorded pyrimidine synthesis may be classified as of this type. Frankland and Kolbe obtained a solid 'cyanalkine' on heating propionitrile with potassium, later investigation by Meyer revealed that the cyanalkines were 4-amino-2,5,6-trialkylpyrimidines (56) (fig. 3), and Frankland and Kolbe's reaction the 2,6-diethyl-5-methyl compound.

c) Route three

The insertion of a single carbon atom between the nitrogens of a 1,3-diamine to obtain a hydrogenated pyrimidine may be achieved by a number of conventional processes. A convenient synthesis of the parent compound pyrimidine (1) proceeds from malondialdehyde diethylacetal (33) and formamide.

Malondiamide and its derivatives have frequently served for the synthesis of both reduced and unreduced pyrimidines. Thus barbituric acids (58) are produced from malonamides (57) with dialkylcarbonates, phosgene, or oxalyl chloride. Somewhat similar is Traube's procedure of allowing a malonyl chloride to react with ethyl carbamate and cyclising the product to the barbituric acid with sodium ethoxide.

Malonic ester or malonyl chloride with malonamides yield 4,6-dihydroxy-2-methylpyrimidines (59). Malondiamidine is analagous, but naturally more facile reactions yield 2-alkyl-4,6-diaminopyrimidines (60) with carboxylic esters; and 4,6-diamino-2-hydroxypyrimidines with chloroformic esters or diethyl carbonate.
Although the method is rather limited in scope, it is especially valuable in that it provides a convenient preparation for 4,6-diaminopyrimidine, a compound difficult to prepare by other methods.

An interesting analogous reaction occurs when urea is condensed with ethyl oxaloacetate. The initial product is the hydantoin which upon vigorous alkaline treatment undergoes a ring opening closure rearrangement to orotic acid, a key compound in the biosynthesis of pyrimidines.

d) A fourth route which is essentially a general synthetic route for uracil and 2-thiouracils was developed by Shaw in 1955. It is best thought of in simple general terms as the cyclisation of an aminomethyleneacylcarbamate such as under mildly alkaline conditions, to give the pyrimidine.

The preparation of the intermediate follows two general routes according to whether a -CN or other group is to appear in the 5-position of the pyrimidine or whether it is to be free.

For example, the preparation of 5-cyano-1-phenyl-3-methyluracil began with the reaction of cyanoacetic acid, ethyl N-methylcarbamate and acetic anhydride which gave ethyl N-methyl-N-cyanoacetylcarbamate. This with ethyl orthoformate gave the ethoxymethylene derivative which on treatment with aniline gave the acyclic ethyl N-(α-cyano-β-anilinoacryloyl)carbamate and thence the pyrimidine. A very similar route led to 5-cyano-1,2'-hydroxyethyluracil and related derivatives.

When dithiourethanes are used in such a synthesis, the reaction takes a slightly different course. Thus the ethoxy-
methylene derivative(71) derived from ethyl N-cyanoacetyldithiocarbamate(70), ring closure occurs to give 5-cyano-2-ethylthio-4-oxo-1,3-thiazine(72). (72) however reacts with, say, methylamine under mild conditions giving first the intermediate(73) and thence, by alkali induced elimination of ethanthiol, 5-cyano-1-methyl-2-thio
turacil(74). An exactly similar reaction sequence has been performed when the cyano group is replaced by p-tolylsulphonyl and similar groups.

When the 5-position is to be free, or occupied by a methyl group (for thymines), the route is different in detail. Thus propiolic anhydride and ethyl carbamate gave ethyl propioloylcarbamate(75). This reacted with aniline vigorously giving (76) which cyclised to 1-phenyluracil(77). An alternative route from ethyl propiolate and ethyl carbamate passed through the carbamate(78). The analogous hydroxymethylethacetylcarbamate(79) also reacts with amines and the resulting analogues of (76) cyclise to give, for example, 6-methyl-1-phenyluracil(80) and several other 1-substituted analogues.

A route to 6-carboxy-2,4-dihydroxypyrimidines (orotic acid(63) and its 3-alkyl derivatives) also follows these general lines. Oxaloacetic acid with ethyl carbamate gave ethoxycarbonylaminomaleic anhydride(81), which with ammonia, gave (82); this was recyclised to the hydantoin(83) and with alkali converted into orotic acid(63). When ammonia is replaced with amines, 3-substituted orotic acids result. By a similar route the 3-phenyl-2-thio derivative of orotic acid has been prepared.

The above methods have been successfully applied to the preparation of nucleosides and are discussed in this context in a later section.
\[(\text{HC} \equiv \text{C}-\text{CO})_2\text{O} + \text{H}_2\text{N} \cdot \text{CO} \cdot \text{OEt} \rightarrow \text{HC} \equiv \text{C}-\text{C}=\text{O} \quad \text{(75)}\]
\[
\begin{align*}
\text{EtO} & \cdot \text{N} \cdot \text{H} \\
\text{Me} & \cdot \text{CH} \cdot \text{CO} \cdot \text{NH} \cdot \text{CO} \cdot \text{OEt}
\end{align*}
\]
\[
\begin{align*}
\text{C}_6\text{H}_5\text{NH}_2 \\
\text{C}_6\text{H}_5\text{NH}_2
\end{align*}
\]
\[
\begin{align*}
\text{EtO} & \cdot \text{N} \cdot \text{H} \\
\text{Me} & \cdot \text{CH} \cdot \text{CO} \cdot \text{NH} \cdot \text{CO} \cdot \text{OEt}
\end{align*}
\]
\[
\begin{align*}
\text{C}_6\text{H}_5\text{NH}_2 & \\
\text{C}_6\text{H}_5\text{NH}_2
\end{align*}
\]

\[
\begin{align*}
\text{EtO} & \cdot \text{N} \cdot \text{H} \\
\text{Me} & \cdot \text{CH} \cdot \text{CO} \cdot \text{NH} \cdot \text{CO} \cdot \text{OEt}
\end{align*}
\]
\[
\begin{align*}
\text{C}_6\text{H}_5\text{NH}_2 & \\
\text{C}_6\text{H}_5\text{NH}_2
\end{align*}
\]

\[
\begin{align*}
\text{EtO} & \cdot \text{N} \cdot \text{H} \\
\text{Me} & \cdot \text{CH} \cdot \text{CO} \cdot \text{NH} \cdot \text{CO} \cdot \text{OEt}
\end{align*}
\]
\[
\begin{align*}
\text{C}_6\text{H}_5\text{NH}_2 & \\
\text{C}_6\text{H}_5\text{NH}_2
\end{align*}
\]
a) Introduction

The limited, but encouraging, success of certain simple purine derivatives and related compounds in clinical tests against human neoplastic diseases, in the early 60's, stimulated the synthesis and study of a vast number of potential purine antagonists. The search for purine analogues, which might exhibit antitumour activity because of interference with the synthesis of the naturally occurring purines, or with their utilisation in nucleic acid biosynthesis, has been divided broadly into two areas; (1) variation in the nature and position of substituent on the intact purine ring and; (2) modification of the ring system itself, usually by replacement of a methylene group (CH=) by nitrogen (N=); or by the preparation of isomeric heterocyclic systems, in which the positions of carbon and nitrogen atoms have been reshuffled.

The first area has been extensively investigated and is the subject of a recent review by R.K. Robins. Much of the research in the second area has been concerned with the preparation of various azapurines, of which 8-aza-guanine and 2- and 8-aza-adenine have been of particular interest.

The deazapurine ring system also occurs naturally; thus the antibiotic Viomycin has been shown by Johnson and co-workers to be a dihydro derivative of a 9-deazapurine. Furthermore, the isolation in 1956 of two new antibiotics, Tubercidin and Toyocamayan and their identification as derivatives of 7-deaza-purine, indicate the effectiveness of such structural modifications on biological activity (see structure 84).
It has been demonstrated that both of these antibiotics are incorporated by the cell into both RNA and DNA (as deoxyribosides) with the dramatic and lethal consequence that RNA, DNA and protein synthesis ceases.

Heterocyclic systems isomeric with the purines have likewise been the subject of considerable recent study, especially the pyrazolo[3,4-d]pyrimidine ring system.

4-Aminopyrazolo[3,4-d]pyrimidine (4APP)(85) has been shown to prolong the life span of leukemic mice, and significantly inhibit the growth of Adenocarcinoma 755. It has also been shown to exhibit a differential inhibitory action upon the growth of malignant cells in the tissue culture. Inhibition of ascites tumor growth by 4APP has also been reported. Henderson and Junda reported that in neoplastic tissue 4APP was isolated in the form of its riboside and mono-, di- and triphosphate derivatives, corresponding to the phosphates of adenosine. Way and Parks and Roy have synthesised the nucleotide of 4APP with purified enzyme systems. Thus it seemed quite possible that 4APP might exert antitumor activity as the nucleoside or nucleotide form.

In order to select a less toxic derivative in this series than 4APP, which had shown signs of hepatotoxicity in man, Robins et al prepared a number of compounds substituted at position 1
or on the 4-amino group, or both. Derivatives of 4-APP with a tetrahydrofuryl or tetrahydropyranyl ring at position 1 were found to be particularly active and can be considered analogues of 4-APP deoxyriboside.

\[
\begin{align*}
\text{(86)} \\
\text{a) } & R = Y = H, \quad X = O \\
\text{b) } & R = H, \quad Y = \text{OH}, \quad X = O \\
\text{c) } & R = Y = H, \quad X = S
\end{align*}
\]

Of special interest also is the hypoxanthine analogue allopurinol(86a), which is a known inhibitor of xanthine oxidase in vivo (and in vitro). This property makes the compound valuable in the treatment of gout and related diseases in which diminished oxidation of purines is required. Other related pyrazolo[3,4-d]pyrimidines including the xanthine analogue (86b) oxyallopurinol and the thiohypoxanthine analogue (86c), thiopurinol, have a similar inhibitory effect on xanthine oxidase. Many compounds of this type have marked antitumor and antileukemic activity.

Studies of the antitumor activity of 2-amino-9-methyl-6-purinethiol by Lepage and Jones stimulated the synthesis of numerous derivatives of 2-amino-6-purinethiol possessing an alkyl substituent at position 9. It was found that a large number of 9-alkyl and 9-alkyl-6-alkylthio derivatives of thioguanine(87a) possess a therapeutic index much superior to the parent compound.

The compound 2-amino-9-n-propyl-6-purinethiol(87b) is especially noteworthy since it possesses a therapeutic index of 64 against Adenocarcinoma 755, whereas thioguanine has a therapeutic index of only 4. It is of considerable interest that
(87b) is also active at a lower dosage than thioguanine. Since Sartorelli and LePage have shown that thioguanine acts at more than one site in purine metabolism, it is quite possible that 9-alkyl derivatives act at only one site. Thus the superior therapeutic indices of a number of these derivatives over that of thioguanine probably result from more selective action of the antitumor drug. According to LePage, the 9-alkylated-2-amino-6-purinethiols appear to have an entirely different mechanism of action from that of thioguanine, for he found that conversion to the nucleotide was a necessary condition for purinethiols to be active as feedback inhibitors. This is in accord with data recorded by Yates and Pardee, whereas it was found that the 9-alkyl derivatives of thioguanine were not converted to the nucleotide form in vivo.

This step would require dealkylation at position 9 to yield the parent thioguanine. With mice bearing Ehrlich and Mecca ascites tumors, LePage and Jones have investigated this possibility and reported that no dealkylation took place.

\[ \begin{align*}
(a) & \quad R = R' = H \\
(b) & \quad R = n\text{-propyl}, R' = H
\end{align*} \]

6-Mercaptopurine exhibits an approximate therapeutic index of 30 against Ad \textit{755} and according to Robins, 2-alkyl-thiopurines exhibit a therapeutic index equal to or greater than that of 6-mercaptopurine in this test system. Evidence has been presented that 6-mercaptopurine acts on tumors via interference
with the biosynthesis of phosphopyridine nucleotide co-enzymes. 

Many different metabolic effects have been observed with the active purines and purine analogues, and inhibition has been shown to occur at various sites in the biochemical sequence of nucleic acid biosynthesis. Undoubtedly, numerous different enzyme systems are involved. It is quite possible however, that enzymes which accept preformed purines and purine nucleosides require certain structural features. Although the binding sites for the natural purines may not be identical, it seems likely that these sites would be restricted. Much has been learned concerning the desirable structural variations on the purine ring which are most likely to result in the selective biological activity. Further refinements in the structure and improvements in the activity of these important compounds and the study of their biochemical relationships to the precursors of nucleic acid and vital co-enzymes is a most exciting challenge. It is abundantly clear from the present biochemical studies, that the purines and purine nucleosides are capable of highly specific action and represent an area which has only begun to be investigated in cancer chemotherapy.
b) Established synthetic routes to the pyrazolo[3,4-d]
pyrimidine ring system

The two most widely used routes to the pyrazolo[3,4-d]
pyrimidine skeleton proceed via initial formation of the pyrazolo
ring and subsequent fusion of a suitable carbon-nitrogen fragment
to give the pyrimidine ring. The first and most popular route
involves the initial formation of the 4-amino-5-cyanopyrazole,
or 4-amino-5-carboxamidopyrazole followed by fusion with a carbon-
nitrogen fragment to yield the substituted pyrazolo[3,4-d]-
pyrimidine.

The first useful synthesis of the pyrazole ring was reported
by Claisen and Haase in 1895. This involved the reaction of
phenylhydrazine and ethoxymethylene malonic ester, and the acyclic
intermediate thus formed, subsequently cyclised by heat to
give the substituted pyrazole.

In 1956 Robins et al used this compound as a precursor for
1-substituted 4-carboxamido-5-aminopyrazole from which, by fusion
with various carbon-nitrogen fragments, e.g. urea, substituted
pyrazolo[3,4-d]pyrimidines were obtained. A much simpler route,
however, was available for the synthesis of the required
4,5-disubstituted and 1,4,5-trisubstituted pyrazole ring. In
1956 Cheng and Robins, by reaction of ethoxymethylene malononitrile
and methylhydrazine, prepared 5-amino-4-cyano-1-methylpyrazole
(91). The general route is given in fig. 4.
Hydrolysis, to carboxamide, of the nitrile group at position 4 with concentrated sulphuric acid gave the required substituted ring structure, 5-amino-1-methylpyrazole-4-carboxamide(92).

Heating 1-alkyl(aryl)-5-amino-4-pyrazolecarboxamides with formamide gave 1-alkyl(aryl)-4-hydroxypyrazolo[3,4-d]pyrimidines (93) and with urea gave 1-alkyl(aryl)-4,6-dihydroxypyrazolo[3,4-d]pyrimidine(94). Phosphoryl chloride and (93) gave the 1-alkyl(aryl)-4-chloropyrazolo[3,4-d]pyrimidines(95) and with (94) gave the 1-alkyl(aryl)-4,6-dichloropyrazolo[3,4-d]pyrimidine(96), which upon alkaline hydrolysis gave the 1-alkyl(aryl)-4-chloro-6-hydroxypyrazolo[3,4-d]pyrimidine(97).

Both (95) and (97) can be utilised for the synthesis of additional 4-substituted pyrazolo[3,4-d]pyrimidines by nucleophilic displacement of the chlorine atom. The preparation of various 1-alkyl(aryl)-4-aminopyrazolo[3,4-d]pyrimidines was accomplished by two routes. The treatment of 1-alkyl(aryl)-5-amino-4-cyanopyrazole(91) with boiling formamide, offered the most direct method of synthesis; the treatment of an o-substituted aminonitrile with formamide, to close the pyrimidine ring, was first applied successfully to the synthesis of 4-aminopyrazolo[3,4-d]pyrimidine(98). However, numerous N-substituted amino derivatives(99), were prepared by the reaction of 4-chloropyrazolo[3,4-d]pyrimidines(95) with various primary and secondary amines.

1-Alkyl(aryl)-4-mercaptopyrazolo[3,4-d]pyrimidines(100) were synthesised by Robins from either the corresponding 4-hydroxy derivative(93) with phosphorous pentasulphide or by treatment...
of the corresponding 4-chloro compounds\(^{(95)}\) in boiling ethanol with thiourea. A synthesis of 6-alkyl-4-hydroxypyrazolo[3,4-d]pyrimidine\(^{(102)}\) was devised by Robins, from the corresponding 5-acylamino-4-cyanopyrazoles. It was found that when 5-amino-4-cyanopyrazoles\(^{(91)}\) were acylated by either acetic or propionic anhydride to give the corresponding 5-acylamino-4-cyanopyrazoles \(^{(101)}\), these derivatives, when treated with hydrogen peroxide in alkaline solution, gave the desired 6-alkyl-4-hydroxypyrazolo[3,4-d]pyrimidines\(^{(102)}\) in excellent yield.

Druey and Schmidt found that reaction of ethyl ethoxymethylene cyanoacetate\(^{(103)}\) and mono-substituted hydrazines gave an alkyl-(aryl)hydrazino ethoxymethyleneacyanoacetate\(^{(104)}\), which underwent an intramolecular rearrangement and cyclised to the 1-alkyl(aryl)-5-amino-4-carbethoxy pyrazole\(^{(105)}\). This, on fusion with formamide, gave the 1-alkyl(aryl)-4-hydroxypyrazolo[3,4-d]pyrimidine\(^{(93)}\).  

During the past decade or so, work by Taylor, and also to a lesser degree by Breukink and Verkade, has demonstrated the utility and versatility of o-aminonitriles as intermediates for the synthesis of condensed pyrimidine heterocycles.

The reaction of 4-cyano-5-aminopyrazole \(^{(91, R = H)}\) with triethyl orthoformate and acetic anhydride gave the intermediate ethoxymethyleneamino derivative\(^{(106)}\), which upon subsequent treatment with a primary amine yielded 4-cyano-5-alkyl(aryl)aminomethyleneaminopyrazole\(^{(107)}\); \(^{(107)}\) underwent a base catalysed intramolecular ring closure to 5-alkyl(aryl)-4-iminopyrazolo[3,4-d]pyrimidine\(^{(108)}\), which in turn rearranged readily, in the presence of a base, to 4-alkyl(aryl)aminopyrazolo[3,4-d]pyrimidine\(^{(99, R, = R_1 = H)}\).
In 1961 Taylor devised a novel synthesis for fused 4-mercaptopyrimidines. Previously available methods for the preparation of these compounds had involved either direct replacement of oxygen by sulphur, through the agency of phosphorous pentasulphide, or of a halogen atom by action of thiourea or an alkali hydrosulphide; or occasionally by suitable ring-closure procedures such as the cyclisation of o-amino thioamides with orthoesters, acetic anhydride-formic acid, acid anhydrides and the closely related cyclisation of o-amino nitriles with sodium sulphide and acid anhydrides.

Taylor reported a convenient, one step synthesis of fused 4-mercaptopyrimidine heterocycles which involved the condensation of o-amino nitriles (109) with thioamides in ethanol saturated with hydrogen chloride. The reaction was believed to be initiated by the addition of the sulphur atom of the thioamide to the protonated nitrile (109a) to give the intermediate (110), which cyclised by loss of ammonia (or amine) to the m-thiazine (111). Addition of alkali to (111) initiated a facile skeletal rearrangement via the ring opening, ring closure sequence pictured to give the stable aromatic anion of the fused 4-mercaptopyrimidine (112). Final acidification of the reaction mixture resulted in the precipitation of the product (113).

A more recent synthesis of these pharmacologically important compounds has been reported, again by Taylor and co-workers. This new and much milder procedure consisted of the reaction of an o-amino nitrile (91, $R = H$) with triethyl orthoformate to give the intermediate ethoxymethylene amino derivative
(106) which, without isolation, is treated with sodium hydro-
sulphide. The fused mercaptopyrimidine(100) is obtained in excellent
yield.

Fused 2-mercaptopuridines have been prepared by the
condensation of an o-amino nitrile with phenyl isothiocyanate
to give N-phenyl-N'-[(o-cyanoaryl)thiourea(114).
(114) cyclised
to give the 2-thio-4-imino fused pyrimidine ring (115), which,
in turn upon boiling under reflux in aqueous DMF, rearranged
to the fused 2-thio-4-anilinopyrimidine compound (116).

No record has been found of this method being used for
the synthesis of 6-mercaptopurazol[3,4-d]pyrimidine, however,
it seems eminently suitable.

The reaction of aromatic and heterocyclic o-amino nitriles
with carbon disulphide in pyridine solution constitutes a
convenient, one-step synthesis of fused 2,4-dimercaptopuridines,
where the only previously available general synthesis had involved
the treatment of a fused dichloropyrimidine with sodium hydro-
sulphide or thiourea. o-Amino nitriles, e.g. 3-amino-4-cyano-
pyrazole(91, R=H) when reacted with carbon disulphide in
pyridine gave a pyridinium salt of the m-thiazine (117). Treat-
ment of (117) with a base in the cold resulted in the
instantaneous and quantitative intramolecular rearrangement to
(118), i.e. 4,6-dimercaptopurazol[3,4-d]pyrimidine.

A number of examples of the base catalysed dimerisation of
the o-amino nitriles, leading to the fused 4-aminopyrimidine
heterocycles have been reported by Taylor. The reaction may be
illustrated by the dimerisation of 2-amino-5-nitrobenzonitrile
(119) to give 2-(2'-amino-5'-nitrophenyl)-4-amino-6-nitroquin-
azoline\(^\text{11}\). It was suggested that this reaction proceeded by initial condensation of the amino group of one molecule of the o-amino nitrile with the nitrile group of a second molecule to give the intermediate amidine\(^\text{12}\), which then underwent a second intramolecular, amine-nitrile condensation to give the observed product.

Extension of this reaction to heterocyclic o-amino nitriles led to a new and useful synthetic route to 6-alkyl(aryl)-4-amino-pyrazolo\([3,4-\text{d}]\)pyrimidines. Dimerisation of, e.g. 5-amino-4-cyanopyrazoles\(^\text{91}\), was possible only under very forcing conditions to give the bicyclic compound \(^\text{122}\).

Such a reaction involved treatment with methanolic ammonia at 200. In contrast to the difficulty experienced in dimerising these two compounds, it was found that mixed condensations with a wide variety of nitriles readily took place to give the required 6-alkyl(aryl)-4-aminopyrazolo\([3,4-\text{d}]\)pyrimidines\(^\text{123}\).

When Shaw first reacted ethyl N-(\(\alpha\)-cyano-\(\beta\)-ethoxyacryloyl)-carbamate\(^\text{124}\) with a monosubstituted hydrazine, e.g. phenylhydrazine, he postulated that a linear aminomethylene derivative \(^\text{125}\) was first formed and that this cyclised to the anilino-uracil\(^\text{126}\) when heated or warmed with alkali, but at the time no spectroscopic evidence supporting these conclusions had been presented. However, reaction of N-acetyl-N'-(\(\alpha\)-cyano-\(\beta\)-ethoxyacryloyl)urea\(^\text{127}\) with phenylhydrazine gave a compound \(^\text{128}\) with no CN absorption in its i.r. spectrum. Mild hydrolysis of the substance gave a product \(^\text{129}\), the elemental analysis of which suggested that an acetyl group had been lost. \(^\text{128}\) and \(^\text{129}\) later proved to have the structures shown.
These results prompted a reinvestigation of the original acryloylcarbamate reaction and it was found that the first reaction product of (124) and phenylhydrazine was indeed the linear amino-methylene derivative (125). However, upon recrystallisation it isomerised to a compound (130) having no CN absorption in the i.r.

When either (125) or (130) was heated at 160° for a few min, ethanol was lost, and another compound (94, R'=C₆H₅) was isolated with no CN band in the i.r., and identical to the material previously regarded as the anilinouracil (126). In addition, the aminopyrazole (129), when heated in pyridine for 10 h, gave the pyrazolo[3,4-d]pyrimidine (94) identical with the compound prepared by cyclisation of (130).

Extensions of this reaction for the synthesis of 1-alkyl-(aryl)-6-alkyl(aryl)-4-hydroxy-; 1-alkyl(aryl)-6-mercapto-4-hydroxy-; and 4,6-dihydroxy-1-β-D-glycosyl derivatives are discussed in the text.
a) Introduction

Work on the nucleic acids, polymeric materials originally isolated from cell nuclei, was initiated in the nineteenth century by Meischer, Altmann and Kessel, and important contributions in the early part of this century were made by Levene and others when the structure of the major bases, and of the sugars was established.

After the Second World War, there were initially two major developments, both of which were greatly helped by the application of new chromatographic techniques. Improved methods of isolation and careful analytical work by Chargraff and others established that nucleic acids were polymers of high molecular weight, and that, in DNA, the ratio of the base content of adenine to thymine, and of guanine to cytosine, was equal to one. At the same time, chemical investigations, mainly by Todd and co-workers, led to the synthesis of nucleosides and nucleotides and the determination of the structure of the nucleotides obtained by chemical and enzymatic hydrolysis of nucleic acids. This made it possible to formulate nucleic acids as 3', 5', - linked polynucleotides. The relationship between the various derivatives is indicated by the following hydrolytic sequence:-

Nucleic acids $\longrightarrow$ Nucleotides
Nucleotides $\longrightarrow$ Nucleosides and orthophosphate
Nucleosides $\longrightarrow$ Base (Purine or Pyrimidine) plus sugar(Ribose or 2-deoxyribose)
With this work as a basis, Watson and Crick were led to their brilliant interpretation of X-ray crystallographic studies of polymeric DNA by Wilkins and others, and to propose the specifically hydrogen bonded double-stranded helical structure for DNA.

The term nucleoside was originally restricted to the purine and pyrimidine N-glycosides of ribose and 2-deoxyribose derived from nucleic acids, but now may be applied to other heterocyclic glycosides including the 5,6-dimethylbenzimidazole riboside from vitamin B₁; and pseudouridine, a C-linked glycoside.

Many nucleosides are useful antibiotics, Cordycepin(131), isolated from the mould Cordyceps militaris is active against strains of B. subtilis and an avian tubercle bacillus. Nebularine (132), isolated from the mushroom Agaricus (clytocybe) nebularis and from the culture media filtrates of a streptomyces, shows activity against mycobacteria and mouse streptomyces. Nucleocidin(133), a streptomyces product, shows phenomenal anti-trypansomal activity as well as possessing broad spectrum antibacterial properties; and Amicetin(134), which is an antitubercular antibiotic produced by streptomyces vinacendsruppus and streptomyces fasciculatis, to mention but a few.

Full structural determination of a nucleoside requires the elucidation of the following points:

a) The structure of the heterocyclic base
b) The structure of the sugar
c) The site of attachment of the sugar to the base.
d) The ring structure of the sugar
e) The configuration of the glycosidic linkage.
b) Established routes in the synthesis of nucleosides

Since the early work of Fischer and Helfrich, considerable effort has been expended on the synthesis of nucleosides, partly as a complementary method to degradation studies for the elucidation of the structure of the naturally occurring compounds (and hence nucleotides and nucleic acids); and partly to provide analogues of value for the interpretations and extensions, of biological action, in terms of chemical structure. In recent years the incentive offered by the isolation of nucleoside anti-biotics, as well as the possible utility of 'abnormal' nucleosides as chemotherapeutic agents against neoplastic diseases, has intensified this effort and led to the rapid development of methods by means of which, almost any nucleoside, natural or unnatural, may be synthesised.

These methods fall into three categories. In the first, a preformed purine or pyrimidine derivative is treated with a suitably reactive form of sugar, commonly a glycosyl halide; secondly the purine or pyrimidine ring system is constructed from a simple N-glycosyl precursor, and in the third, a preformed nucleoside is modified, either in the sugar moiety or in the purine or pyrimidine base. All three routes have been used with success for the synthesis of naturally occurring nucleosides, and all three have their biochemical analogies. A possible fourth method, fabrication of the sugar on a simple N-alkyl(or acyl) purine or
pyrimidine base has not been observed in metabolic processes nor has it been used in a chemical sense.

CLASS 1 Coupling of sugars with a preformed base

The earliest work on nucleoside synthesis is that of Emil Fischer and co-workers who used a method that, with a number of modifications and refinements, has had wide application. In this classical approach an appropriate silver purine was condensed with an acetohalogenosugar and the product deacetylated. The treatment of silver 2,8-dichloroadenine (135) (or 2,6,8-trichloropurine) with acetobromoglucose gave the 9-glucosyl compound (136) though the position of the glycosyl linkage was not established until much later. Dehalogenation of 2,8-dichloro-9-glucosyladenine gave 9-D-glucopyranosyladenine (137).

Application of the same method to the synthesis of pyrimidine nucleosides by the treatment of a silver pyrimidine with an acetohalogen sugar was less successful, since with pyrimidines containing a lactam-lactim tautomeric system either an O-glycoside was obtained (e.g. with uracil), or else no coupling occurred (e.g. with cytosine). However, by restricting this phototropic change through the use of 2,6-dialkoxypyrimidines, Hilbert and Johnson were able to prepare glycosyl pyrimidines that, by analogy with the product from methyl iodide and 2,6-diethoxypyrimidine, were N'-glycosyl derivatives. Thus treatment of 2,6-diethoxypyrimidine (138) with acetobromoglucose, gave a product from which the ethyl and acetyl groups could be removed by methanolic hydrogen chloride, to yield 1-D-glucofuranosyluracil (139), or by methanolic
ammonia to give 1-D-glucofuranosylcytosine(140).

Numerous glycopyranosylpyrimidines were prepared this way, but the method could not be applied to the synthesis of naturally occurring nucleosides, until development of the required ribofuranosyl halide. In 1947, Howard, Lythgoe and Todd, obtained cytidine, (and hence uridine by deamination) starting from acetobromoribofuranose and 2,6-diethoxypyrimidine.

These approaches, while representing the first synthesis of natural ribonucleosides, were somewhat limited in that low yields were generally obtained, and also they could not be applied to purines containing an amino group basic enough to react with the glycosyl halide. Prior acetylation of the amino group and use of chloromercury purines which have greatly increased yields, have also meant that this method has been widely used as a route to naturally occurring nucleosides, as well as a host of analogues, among these being, 1- and 2-β-D-ribofuranosyl-pyrazolo[3,4-d]pyrimidines, analogues of which are discussed in the text.

That the stereochemistry of the glycosyl linkage in this type of synthesis is controlled by the 2'acyloxy group of the sugar moiety was recognised in a most useful rule proposed by Baker and his co-workers in 1954. This stated that condensation of a heavy metal salt of a purine(or pyrimidine) with an acetylated glycosyl halide would form a nucleoside with a C1-C2-transconfiguration in the sugar moiety, regardless of the original configuration at C1 - C2. If the haledeno sugar has the 1,2 cis configuration, the purine will enter by a single $S_N$ 2 reaction with Walden inversion at C1, giving a nucleoside with C1-C2 transconfiguration and which may be α or β depending on the sugar (see fig.5).
Halogeno sugars with a 1,2-trans-configuration would undergo two $S_N^2$ reactions, with two Walden inversions, the first of which is intramolecular, with participation of the 2-acyloxy group.

Thus formation of $\beta$-ribosides from $\beta$-ribose derivatives is seen to depend on the neighbouring group participation of the 2-acyloxy group. If such a group is missing or sterically hindered a mixture of products is expected. This was indeed found to be true in the adenosine synthesis using $5'$-O-benzoyl-0-ribofuranosylbromide-2,3-cyclic carbonate which gave both natural ($\beta$) adenosine and its $\alpha$-anomer.

Although earlier approaches to direct coupling of metal pyrimidine and sugar to form $N$-glycosides failed, Fox and co-workers were able to extend the chloromercury method to the synthesis of pyrimidine nucleosides, in spite of possible lactim-lactam tautomerism.

Condensation of dithyminylmercury with an acylated sugar halide followed by removal of protecting groups gave $N'$-glycosyl pyrimidines in good yield. In this way 1-$\beta$-D-ribofuranosylthymine and the analogous 1-$\beta$-D-xylofuranosyl derivatives were prepared. Determination of the configuration of the products showed that the trans rule was also applicable to the syntheses of $N$-glycosylpyrimidines.

**CLASS 2 Construction of nucleosides from simple glycosyl derivatives**

This type of synthesis, first successfully developed by Todd, provided unambiguous evidence for the position of the glycosyl residue in the natural purine nucleosides and, as a general method, was used for the preparation of a number of 9-glycosylpurines,
including adenosine. In the particular approach that was used, purine nucleosides were formed from pyrimidylglycosylamines by completion of the imidazole ring.

Treatment of 4,6-diamino-2-methylthiopyrimidine (141) (the 2-methylthio substituent increases the reactivity of the aminopyrimidine) with 2,3,4-tri-O-acetyl-5-O-benzyl-D-ribose (142) gave a Schiff base (143) which on deacetylation rearranged to the 5'-O-benzyl-D-ribofuranosyl derivative (144). This was coupled with diazotised 2,5-dichloroaniline (145) and the product acetylated and reduced with zinc and acetic acid to the 5-amino compound (146), which was then thioformylated with dithioformic acid (147). The resulting intermediate was cyclised, the product (148) reacetylated and the 2-methylthio and 5'-benzyl groups removed. Deacetylation then gave adenosine, (149), not unexpectedly, in rather low yield.

Interaction of 2,3,5-tri-O-acetyl-D-ribofuranosyl chloride and silver 4,5-dicarbomethoxyimidazole (150) gave a product (151), which, on treatment with ammonia, yielded 1-D-ribofuranosylimidazole-4,5-dicarboxamide (152). A modified Hofmann reaction with alkaline hypobromite effected ring closure of (152) to xanthosine (153).

Ring closure of a glycosylamine derivative has been used by Shaw et al to prepare pyrimidine nucleosides. Various 5-cyano-3-D-glycosyluracils were obtained by the reaction of D-glycosylamines with ethyl N-(α-cyano-β-ethoxyacryloyl)carbamate (124) and ethyl N-(α-cyano-β-ethoxy-N-methylacryloyl)carbamate (67). This reaction gave a product (154) which failed to give a precipitate with basic lead acetate; however, when a mildly alkaline solution was warmed for a short time and then cooled, an insoluble lead salt appeared. This behaviour suggested the formation of a linear
compound(154) and subsequent cyclisation of this to a pyrimidine nucleoside(155).

Uridine(158) was synthesised in a similar fashion from β-ethoxy-N-ethoxycarbonylacrylamide(78) and 2,3,5-tri-O-benzoyl-D-ribofuranosylamine(157). The furanose glycosylamine was synthesised by catalytic hydrogenation of 2,3,5-tri-O-benzoyl-β-D-ribofuranosylazide(156), obtained from the ribosyl halide and sodium azide in methyl cyanide. This method is an extension of one used by Bertho for the preparation of 2,3,4,6-tetra-O-acetylglucosylazide and D-glucosamine.

From its method of preparation, the ribosylamine(157) would formally be expected to have the β-configuration. However, the isolation of α- and β-glycyl derivatives of the amino sugar suggests that mutarotation occurs readily and that the ribosylamine is perhaps best regarded as an α-, β-mixture.

Reaction of the ribosylamine with the acrylamide in the presence of triethylamine, alkali induced cyclisation and debenzoylation of the product with methanolic sodium methoxide gave uridine(158).

An analogous reaction of the tri-O-benzoyl-D-ribofuranosylamine with β-ethoxyacryloylisothiocyanate(159) or, β-methoxy-α-methylacryloylisothiocyanate yielded 2-thiouridine(160) (converted into uridine by the action of aqueous chloroacetic acid) or 5-methyl-2-thiouridine respectively, after removal of the benzoyl groups.

In all cases only β-nucleoside was obtained, none of the isomeric α-compounds being detected, even when a known α-β-glycosylamine mixture was used. It is likely that pyrimidine nucleoside formation via linear precursors of the type (161), follows
a general rule to give 1,2-trans-configuration at the glycosyl linkage.

Molecular models suggest that the β-linear forms should cyclise more readily than the α-anomers in which steric hindrance of the NH group by the 2'-O-benzoyl group occurs.

An alternative synthesis of 5-amino-1-β-ribofuranosylimidazole-4-carboxamide also makes use of a linear ribofuranosylamine derivative. Reaction of the imino-ether, ethyl N-(carbamoylcyanomethyl)formimidate (162), (prepared from ethyl formimidate hydrochloride and α-amino-α-cyanoacetamide) with 2,3,5-tri-O-benzoylribofuranosylamine and debenzoylation of the product gave 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide (163), the structure of which was established by conversion of the compound into inosine, on treatment with acetic anhydride and formic acid.

The synthesis of several 1-D-glycosyl derivatives of 6-aminouracil by a Traube reaction of O-acylglycosyl ureas with cyanoacetic acid, has been reported.

A related approach has been used for the synthesis of thymidine (167). Urea was condensed with 3,5-di-O-benzoyl-2-deoxy-D-ribofuranose and the resultant deoxyribofuranosyl urea (164) treated with β-ethoxy-α-methylacryloyl chloride (165) to give 1-(β-ethoxy-α-methylacryloyl)-3-(3',5'-di-O-benzoyl-2'-deoxy-D-ribofuranosyl)urea (166). Cyclisation and debenzoylation then yielded the free deoxynucleoside (167).

A new facile preparation of stable glycofuranosylamines reported by Cusack, Rugg and Shaw has much simplified and improved the synthesis of pyrimidine nucleosides.
\[
\begin{align*}
R &= H \text{ or } Me \\
R' &= D-\text{glycosylamine}
\end{align*}
\]
Reaction of the readily available glycopyranosylamine (prepared from the sugar and methanolic ammonia) with acetone, 2,2-dimethoxypropane, and toluene-p-sulphonic acid yields the 3,5-O-isopropylidene glycopyranosylamine tosyl salt. An example is 3,5-O-isopropylidene-β-D-xylofuranosylamine toluene-p-sulphonate(168).

Reaction of the liberated free amine with α-cyano-β-ethoxy-N-ethoxycarbonylacrylamide followed by mild acid hydrolysis to remove the isopropylidene group provides a much simplified route to 5-substituted pyrimidine nucleosides.

A similar reaction sequence, discussed later in this thesis, was used by the author, to prepare uridine by a new route involving the preparation, hydrolysis and decarboxylation of 5-ethoxycarbonyl-uridine.

CLASS 3 Alteration of base or sugar moieties of preformed nucleosides

Inversion of a sugar hydroxyl group via cyclonucleoside formation was first observed with 0₁,3'₁-cyclothymidine which, on alkaline hydrolysis, gave 1-β-D-2'-deoxyfuransylthymine, fig. 6. Subsequently, similar results were obtained with ribonucleosides. As a general approach, the formation of 0₁,2'₁-cyclonucleosides and subsequent ring opening, is of considerable value for the synthesis of glycosyl anomers that, because of the C₁-C₂ trans rule, would not be readily obtained by previously described methods. In this way spongouridine, 1-β-D-arabinofuranosylthymine, 1-β-D-arabinofuranosyl-5-fluorouracil and 1-β-D-lyxofuranosylthymine were prepared from suitable sulphonyl-ribosyl(or xylosyl) derivatives, (see fig. 7).

More conveniently 1-β-D-lyxofuranosyluracil has been obtained by boiling an aqueous solution of 3',5'-di-o-methanesulphonyl-0₂'-cyclouridine (readily obtained by treatment of 2',3',5',-
tri-O-methanesulphonyluridine with one equivalent of sodium hydroxide) or 5'-O-benzoyl-3'-O-methanesulphonyl-02'-cyclouridine. The reaction sequence involved the formation of 3'-O-methanesulphonyl-1-ß-D-arabinofuranosyluracil and O²,3'-cycloxylofuranosyluracil intermediates. The latter was hydrolysed under acidic conditions (resulting from liberation of methanesulphonic acid) to lyxofuranosyluracil derivatives (see fig. 8).

Treatment of 2',3',5'-tri-O-methanesulphonyluridine(169) with three moles of sodium hydroxide yielded the nucleoside epoxide(170). Similarly, acidic hydrolysis of 5'-O-benzoyl-3'-O-methanesulphonyl-O²-2'-cyclouridine(171), gave the benzoyl nucleoside(172), which on treatment with ammonia yielded the epoxide(173) i.e. 5'-O-benzoyl-2',3'-anhydro-1-ß-D-lyxofuranosyluracil, removal of the benzoyl group, followed by amination gave the aminonucleoside 3'-amino-3'-deoxy-1-ß-D-arabinofuranosyluracil(174).

Apart from their use for specific inversion of sugar hydroxyl groups, pyrimidine O²,2'-cyclonucleosides have proved strikingly successful as intermediates in the synthesis of 2'-deoxynucleosides. Treatment of 5'-O-acetyl-2'-O-toluene-p-sulphonyluridine(175), with sodium iodide gave 5'-O-acetyl-2'deoxy-2'iodouridine(177), from which 2'-deoxyuridine(178) was obtained by hydrogenation and deacetylation. The same iodo derivative was formed by the action of sodium iodide and acetic acid on 5'-O-acetyl-0²,2'-cyclouridine(176). This cyclonucleoside was probably an intermediate in the replacement of the tosyl group by iodine, the final product being the result of two displacements with inversion at C₁.

With glycosylpurines, stereochemical considerations preclude the formation of cyclonucleosides involving 2'-hydroxyl groups, and
in any case, cleavage of the purine ring would presumably occur as with the N³,5'-cyclonucleoside salts. Baker and co-workers, however, applied reactions analogous to those involved in cyclonucleoside formation, with considerable effect, to the synthesis of isomers of the aminonucleoside, from puromycin. Methane-sulphonylation of 6-dimethylamino-9-(3'-acetamido-3'-deoxy-α-D-arabinofuranosyl)-purine(179) gave the dimesylate(180), which on treatment with sodium acetate was converted into the α-ribofuranosyl derivative(182). Inversion at C¹ occurred by attack of the 3'-acetamido group with intermediate formation of the oxazoline(181).

The β-arabinofuranosylamino nucleoside was obtained from 2-methylthio-6-dimethylamino-9-β-D-xylofuranosylpurine. Methanesulphonylation of the 3',5'-isopropylidène derivative(183) gave (184), which by removal of the isopropylidene group gave a 2'-O-methanesulphonyl nucleoside(185); (185) was converted into the 2',3'-anhydro-D-lyxosyl compound(186) by treatment with sodium methoxide. Ring opening with ammonia followed by desulphurisation then gave 6-dimethylamino-9-(3'-amino-3'-deoxy-β-D-arabinofuranosyl)purine(187).

This last example demonstrates the use of epoxides for changing a xylose to an arabinose configuration, and also provides a route of value in the synthesis of 3-deoxy nucleosides. The synthesis of 2-deoxy nucleosides, however, requires a more complex reaction sequence. Chloromercury-6-benzamidopurine(188) was condensed with the glycofuranosyl chloride(189) and the product (190) treated with sodium methoxide to remove three acyl groups and form the epoxide 6-amino-9-(2',3'-anhydro-β-D-ribofuranosyl)purine(191). (191) was treated with sodium ethylmercaptide to give the 3'-deoxy-3'-ethylthioxylo-furanosyl derivative(192), which on reaction with thionyl chloride
and sodium bicarbonate yielded 6-amino-9-(3'-chloro-2',3'-dideoxy-2'-ethylthio-β-D-arabinofuranosyl)purine (193). Acetolysis of (193) gave the 2'-deoxy-2'-ethylthio compound (194), which, upon desulphurisation yielded 2'-deoxyadenosine (195).

Modification of substituents in the purine or pyrimidine base is also used as a route to new compounds. Thiation of purine bases yielding 6-mercaptapurines provides a simple route to compounds of possible importance as antitumor agents and bacterial growth antagonists. Treatment of 2',3',5'-tri-O-benzoylinosine (196) with phosphorous pentasulphide gave the 6-mercapto analogue (197), and treatment with methyl iodide yielded the 6-methylmercapto derivative (198).

Chlorination of 2',3',5'-tri-O-acetylpurine nucleosides has given the corresponding 6-chloro derivatives from which the 6-thio compounds were obtained by treatment with sodium thioacetate. Recovery of the 6-thio into the 6-chloro compounds was readily achieved by treatment with chlorine at low temperatures.

Treatment of thio nucleosides with alcoholic ammonia yielded the amino derivative, and with other suitable reagents gave the 6-alkylamino, hydrazino, hydroxylamino and other analogues.

The reaction of uracil nucleosides with formaldehyde, as with other electrophilic reagents, is selective for C₅ of the pyrimidine ring. Treatment of uridine (158) with formaldehyde in the presence of hydrochloric acid gave the 5-hydroxymethyl compound (199), which on hydrogenation yielded 1-β-D-ribofuranosylthymine (200). Condensation of the 5-hydroxymethyl nucleosides (199) with acids and alcohols occurred readily giving, for example, the ester (201), and catalytic oxidation of (199) gave the 5-formyl derivative (202).
Related procedures have been developed for the aminomethylation and chloromethylation of pyrimidine nucleosides and deoxynucleosides. Thus the reaction of uracil with morpholine and formaldehyde gave 5-morpholinomethyluracil; 5-chloromethyluracil is obtained by an analogous treatment with formaldehyde and hydrochloric acid.
A discussion of the synthesis and attempted synthesis of some substituted uracils, pyrazolo[3,4-d]pyrimidines and their glycopyranosyl and glycofuranosyl derivatives.
The synthesis of 1-alkyl(aryl)uracils unsubstituted in the 5-position

The interest generated in substituted pyrimidine compounds by the usefulness of some pyrimidines, described earlier, as chemotherapeutic agents, prompted us to investigate an alternative route to the pyrimidine skeleton(203), in which C₅ of the pyrimidine ring was unsubstituted. Electrophilic substitution in the pyrimidine ring is selective for the C₅ position, and thus the skeleton(203) would be capable of further modification if required.

The established methods for pyrimidine synthesis, as outlined in the introduction, required the prior synthesis of specific carbon and carbon-nitrogen fragments. Any variation in the nature of substituents on nitrogens 1 and 3 would of necessity require other specific fragments to be produced.

We have attempted to devise a synthetic route whereby a preformed acyclic intermediate reagent might condense with a simple primary amine giving an acyclic amine derivative. This in turn could be induced to undergo a condensation cyclisation reaction to give a 5-substituted uracil. The nature of the 5-substituent being such as to allow facile removal, leaving the 5-position free. The reagent produced to fulfil this requirement was ethyl N-(α-ethoxycarbonyl-β-ethoxyacryloyl)-carbamate(205).

Diethyl malonate was hydrolysed to the monopotassium salt with potassium hydroxide in alcohol. The dipotassium salt was
removed, being insoluble in alcohol. Potassium ethyl malonate separated from hot alcohol in high yield (82 %). Ethyl hydrogen malonate was obtained by neutralisation of the weak salt, with concentrated hydrochloric acid. The product, after extraction with ether and vacuum distillation, was obtained as a liquid (96 %).

When refluxed with ethyl carbamate in acetic acid solution, ethyl hydrogen malonate yielded crystals of ethyl N-ethoxycarbonylacryloyl-carbamate (204), which when treated with triethyl orthoformate in acetic anhydride gave ethyl N-\(\alpha\)-ethoxycarbonyl-\(\beta\)-ethoxyacryloyl-carbamate (205) (m.p. 159). Elemental analysis of (205) confirmed its molecular formula to be \(C_{11}H_{14}N_{4}O_{6}\).

Reaction of the acrylamide (205) with methanolic ammonia gave a white precipitate, m.p. 164° (resolidified). Mass spectroscopy indicated a mass ion of 230 which corresponded to that required for the acyclic derivative ethyl N-\(\alpha\)-ethoxycarbonyl-\(\beta\)-amino-acyrlyl-carbamate (206). A subsidiary peak at 184 was present which corresponded to the loss of ethanol, as would be expected if cyclisation to the uracil (207) had occurred on the probe.

Full cyclisation to 5-ethoxycarbonyluracil (207), was achieved by warming an alcoholic solution of the acyclic compound with sodium ethoxide. A white gel resulted which redissolved on neutralisation. This solution gave 5-ethoxycarbonyluracil (207) as an amorphous solid. Recrystallisation gave plates (m.p. 287°), elemental analysis of which confirmed the molecular formula of \(C_{9}H_{8}N_{4}O_{4}\) corresponding to that required for the uracil (207).

Alkaline hydrolysis of (207) with hot sodium hydroxide gave the sodium salt of the parent acid, which in turn gave
5-carboxyuracil (208) on neutralisation with mineral acid. The hydrolysis was followed by titration studies as detailed in fig. 9 and proved to be rather sluggish.

Alkaline hydrolysis of the acyclic carbamate (206) with hot sodium hydroxide also gave 5-carboxyuracil (208), identical to the sample prepared by hydrolysis of 5-ethoxycarbonyluracil. Titration studies of this reaction indicated (fig. 10) that complete hydrolysis occurred certainly within fifteen minutes and probably within the first minute or so. During the reaction the sodium salt of 5-carboxyuracil separated as an amorphous white solid m.p. 350, which dissolved in hot water. Neutralisation of this solution gave 5-carboxyuracil (208) as a white precipitate (m.p. 223). Elemental analysis confirmed the formula to be the required C₅H₄N₂O₄. Mass spectrometry showed two main peaks at 156 and 112. C₅H₄N₂O₄ required 156, and 112 corresponded to the loss of CO₂ (i.e. 44), indicating that decarboxylation of the acid had occurred on the probe to give the uracil (2).

Several methods of decarboxylation of (208) were attempted. The simplest, namely heating the sample, required a temperature of at least 280 before any decomposition occurred. At temperatures above 280, carbon dioxide was evolved easily, leaving a product identical to an authentic sample of uracil (2). However, the object of developing this section was to devise an easy route by which 5-unsubstituted uracils might be prepared, and although uracil itself is stable at 280, many of the proposed uracils, especially the uracil nucleosides would not be stable. A much lower decarboxylation temperature was therefore required.

To this end a number of decarboxylation techniques were
Fig. 9  1 ml Aliquots of alkaline 5-ethoxycarbonyluracil(207) titrated against standard sulphuric acid.

<table>
<thead>
<tr>
<th>Time</th>
<th>Titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>12.8 ml</td>
</tr>
<tr>
<td>5 min</td>
<td>11.2 ml</td>
</tr>
<tr>
<td>10 min</td>
<td>10.4 ml</td>
</tr>
<tr>
<td>15 min</td>
<td>9.8 ml</td>
</tr>
<tr>
<td>25 min</td>
<td>9.2 ml</td>
</tr>
<tr>
<td>35 min</td>
<td>9.0 ml</td>
</tr>
<tr>
<td>40 min</td>
<td>8.8 ml</td>
</tr>
<tr>
<td>50 min</td>
<td>8.6 ml</td>
</tr>
<tr>
<td>60 min</td>
<td>8.6 ml</td>
</tr>
</tbody>
</table>

Fig. 10  1 ml Aliquots of alkaline ethyl N- (\(\alpha\)-ethoxycarbonyl-\(\beta\)-aminoacryloyl) carbamate(206), heated to reflux and titrated against standard sulphuric acid.

<table>
<thead>
<tr>
<th>Time</th>
<th>Titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>32.2 ml</td>
</tr>
<tr>
<td>1 min</td>
<td>23.0 ml</td>
</tr>
<tr>
<td>5 min</td>
<td>22.8 ml</td>
</tr>
<tr>
<td>15 min</td>
<td>22.0 ml</td>
</tr>
<tr>
<td>30 min</td>
<td>22.0 ml</td>
</tr>
</tbody>
</table>
attempted, as detailed below.

a) **Using quinoline and copper powder**

When added to a quinoline solution of 5-carboxyuracil, maintained at 190-210°, copper powder caused the evolution of carbon dioxide to occur. Gaseous evolution ceased after about 1 h., and, on cooling, the solution was made alkaline, ether extracted and the aqueous phase was neutralised with mineral acid. Extraction of this solution with ethyl acetate gave a yellow product which proved to be a mixture of the starting material and uracil. The latter was identical to an authentic sample of uracil.

b) **Using a copper salt**

An intimate mixture of 5-carboxyuracil and a basic copper salt (basic copper carbonate) was heated. Decarboxylation of the acid occurred, however, the minimum temperature required was not significantly below that required for the pure acid.

c) **Using N,N-dimethylaniline**

A solution of the carboxyuracil(208) in N,N-dimethylaniline was heated. Gaseous evolution commenced at 150-160° and was quite rapid at 190°. When all gaseous evolution had ceased, the solution, on cooling, gave crystals which were identical to an authentic sample of uracil.

d) **Using N-ethylmorpholine**

Decarboxylation occurred readily when a suspension of the carboxyuracil in N-ethylmorpholine was maintained at 120-130°. The suspension dissolved and precipitation from the cooled solution with ether gave uracil(2), which proved to be identical to an authentic sample.
This latter method proved to be readily applicable to the decarboxylation of several substituted 5-carboxyuracils, particularly N-nucleosides, and offers a valuable route to uracil nucleosides via their 5-carboxylic acids.

Ethyl N-(\(\alpha\)-ethoxycarbonyl-\(\beta\)-ethoxyacryloyl)carbamate (205) reacted readily with many primary amines to give acyclic derivatives analogous to (206). Methylamine with (205) gave the acyclic compound ethyl N-(\(\alpha\)-ethoxycarbonyl-\(\beta\)-methylaminoacryloyl)carbamate (209). The mass spectrum of (209) showed two peaks. The first at 244 corresponded to the required formula for (209), and the second at 198 suggested loss of ethanol and hence cyclisation on the probe.

The carbamate (209) was treated with sodium ethoxide which initiated a cyclisation reaction to give the uracil ester, 1-methyl-5-ethoxycarbonyluracil (210).

Hydrolysis of 1-methyl-5-ethoxycarbonyluracil (210) to the corresponding carboxylic acid (211) with aqueous sodium hydroxide, which was followed by titration studies, proved to be rather sluggish, but ultimately gave the acid (211). In contrast, treatment of the acyclic intermediate (209) with aqueous sodium hydroxide induced hydrolysis and much more rapid cyclisation to the same 5-carboxyuracil (211). Hydrolysis of the acyclic acrylamide (209) was found to be completed in under ten minutes, whereas complete hydrolysis of the uracil ester (210) was found to require 1h, at room temperature (fig. 13).

An investigation of the various decarboxylation techniques used earlier, gave results similar to those obtained for 5-carboxyuracil (208). 1-Methyl-5-carboxyuracil (210) may be decarboxylated by heating the solid to 260-280°. This carboxyuracil was also readily decarboxylated when heated with quinoline and activated copper powder at
with N,N-dimethylaniline at 160-180°, or (best) with N-ethylmorpholine at 130°. In each case the product gave crystals from nitromethane, identical with an authentic sample of 1-methyluracil(212).

Similarly, the ethoxyacrylamide(205) gave with aniline, benzylamine, phenylethylamine, and furfurylamine, the corresponding amino-carbamates. Namely, ethyl N-(α-ethoxycarbonyl-β-anilinoacryloyl)-carbamate(213); ethyl N-(α-ethoxycarbonyl-β-benzylaminoacryloyl)-carbamate(214); ethyl N-(α-ethoxycarbonyl-β-phenylethylaminoacryloyl)-carbamate(215) and ethyl N-(α-ethoxycarbonyl-β-furfuryl aminoacryloyl)-carbamate(216).

Ethyl N-(α-ethoxycarbonyl-β-anilinoacryloyl)carbamate(213) was obtained as a crystalline precipitate from benzene, and cyclisation to the uracil ester(217) was accomplished by reaction with sodium ethoxide in ethanol. 1-Phenyl-5-ethoxycarbonyluracil(217) was hydrolysed to the corresponding acid(218) with aqueous sodium hydroxide maintained at room temperature for 1 h.

Treatment of the acyclic aminoacrylamide(213) with warm aqueous sodium hydroxide, followed by immediate neutralisation with mineral acid, gave a precipitate of 1-phenyl-5-ethoxycarbonyluracil(218). This precipitate effervesced readily with aqueous sodium bicarbonate solution, confirming its acidic nature, and showed two main peaks in its mass spectrograph. The first peak at 232 was in agreement with the required mass ion for the formula C_{11}H_{11}N_{2}O_{5} of (218). The second peak at 188 indicated decarboxylation to have occurred on the probe.

1-Phenyl-5-carboxyuracil(218), readily decarboxylated at temperatures above 270°, or with N-ethylmorpholine at 120° for 15 min, during which time evolution of carbon dioxide was rapid. 1-Phenyluracil (77) was recovered from the cooled suspension and gave plates (m.p. 321°) from ethyl acetate. Elemental analysis of this purified material
confirmed its molecular formula to be that required for 1-phenyluracil.

Ethyl N-(α-ethoxycarbonyl-β-benzylaminoacryloyl)carbamate(214) was initially obtained as a yellow oil, from the reaction between benzylamine and the ethoxycarbonylacrylamide (205). This oil formed an amorphous solid over several days. Mass spectroscopy showed peaks corresponding to the acyclic carbamate (214) at 320, and the cyclised derivative at 274.

The addition of ethanolic sodium ethoxide to the acyclic acrylamide (214) caused cyclisation to the uracil ester, 1-benzyl-5-ethoxycarbonyluracil (219) to occur. Elemental analysis of this material was in agreement with that required for the structure as shown (i.e. C_{14}H_{14}N_{2}O_{5}).

Phenylethylamine with acrylamide (205) gave a strongly coloured green solution, from which ethyl N-(α-ethoxycarbonyl-β-phenylethylaminoacryloyl)carbamate (215) was precipitated as an oil, upon dilution with water. With cooling and scratching this oil gave crystals of the acyclic acrylamide (215) which, upon treatment with 2 M sodium hydroxide at 50° for 2 min., hydrolysed and cyclised to give 1-phenylethyl-5-carboxyuracil (221), which was recovered as an amorphous white precipitate by neutralisation of the alkaline solution with mineral acid. The mass spectrum of this material showed the required mass ion at 260, and a second peak at 216 suggesting again that decarboxylation occurred on the probe.

The addition of sodium ethoxide to the original green solution of the acyclic acrylamide (215) resulted in the immediate precipitation of a white solid, thought to be the sodium salt of the acyclic acrylamide (215a). This redissolved on standing, and neutralisation of this solution gave 1-phenylethyl-5-ethoxycarbonyluracil (220) as an amorphous solid which gave the required mass ion of 208.
The alkaline hydrolysis of the uracil ester (220) was followed by titration studies (fig. 11), the solution being heated under reflux. It was found that a reaction time in excess of 1h was required for complete hydrolysis to occur. The product was recovered by neutralisation of the cooled solution, and was found to be identical to the sample of 1-phenylethyl-5-carboxyuracil (221) prepared by the action of sodium hydroxide on the acyclic acrylamide (215).

Similarly furfurylamine, when reacted with the acrylamide reagent (205) produced a deeply coloured solution from which a sticky solid was precipitated on dilution with water. Dissolution of this acyclic carbamate (216) in aqueous sodium hydroxide with warming for 2-3 min., followed by neutralisation, gave 1-furfuryl-5-carboxyuracil (223) as an amorphous white solid. This material caused aqueous sodium bicarbonate to effervesce and its structure was confirmed by elemental analysis and mass spectroscopy.

Ethanolic sodium ethoxide when added to the initial solution of the acyclic intermediate (216), caused the precipitation of a white solid, thought to be the sodium salt of this intermediate (216a), which redissolved on standing. 1-Furfuryl-5-ethoxycarbonyluracil (222) was recovered from this solution on neutralisation.

Alkaline hydrolysis of (222) was again followed by the titration of aliquots of the solution, heated under reflux, with standard sulphuric acid (fig. 12). The time required for complete hydrolysis of the ester was again found to be in excess of 1h. Neutralisation of the final solution gave 1-furfuryl-5-carboxyuracil (223), identical with sample prepared directly from the acyclic compound (216).
Fig. 11 1 ml Aliquots of alkaline 1-phenylethyl-5-ethoxycarbonyluracil(220), heated to reflux and titrated against standard hydrochloric acid.

<table>
<thead>
<tr>
<th>Time</th>
<th>Titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>6.2 ml</td>
</tr>
<tr>
<td>10 min</td>
<td>5.5 ml</td>
</tr>
<tr>
<td>20 min</td>
<td>5.2 ml</td>
</tr>
<tr>
<td>30 min</td>
<td>5.1 ml</td>
</tr>
<tr>
<td>40 min</td>
<td>5.0 ml</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time</th>
<th>Titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 min</td>
<td>4.9 ml</td>
</tr>
<tr>
<td>60 min</td>
<td>4.9 ml</td>
</tr>
<tr>
<td>120 min</td>
<td>4.6 ml</td>
</tr>
<tr>
<td>420 min</td>
<td>4.6 ml</td>
</tr>
</tbody>
</table>

Fig. 12 1 ml Aliquots of alkaline 1-furfuryl-5-ethoxy-carbonyluracil(222), heated at reflux and titrated against standard sulphuric acid.

<table>
<thead>
<tr>
<th>Time</th>
<th>Titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>10.5 ml</td>
</tr>
<tr>
<td>5 min</td>
<td>9.3 ml</td>
</tr>
<tr>
<td>15 min</td>
<td>8.4 ml</td>
</tr>
<tr>
<td>30 min</td>
<td>7.9 ml</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time</th>
<th>Titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>45 min</td>
<td>7.6 ml</td>
</tr>
<tr>
<td>60 min</td>
<td>7.4 ml</td>
</tr>
<tr>
<td>105 min</td>
<td>7.3 ml</td>
</tr>
<tr>
<td>165 min</td>
<td>7.3 ml</td>
</tr>
</tbody>
</table>
Several points of interest arise from the reaction sequences described above, which may be of use in the synthesis and identification of 1-ß-D-uracil nucleosides.

a) The relative ease with which the 5-carboxyuracils are formed by alkaline hydrolysis of the acyclic aminoacrylamides, as compared with their formation by hydrolysis of the uracil esters.

b) The increased ease with which the 5-carboxyuracils decarboxylate in more basic solvent systems.

c) The characteristic u.v. absorptions and absorption shifts exhibited in acid and alkaline solutions.

a) 5-Carboxyuracils: Preparation from acyclic carbamate and uracil 5-carboxylic acid esters.

No simple relationship exists between the hydrolysis conditions required for the acyclic carbamate, and those required for the ester uracil. The time taken, to complete the hydrolysis of the acyclic compound is however, in each case significantly less than that required by the preformed uracil (fig. 13).

The base catalysed cyclisation of the acyclic carbamate to the uracil using sodium ethoxide, is a reasonably fast reaction. In view of this, it is reasonable to assume that the order of reactions for the acyclic carbamate is, first hydrolysis and then cyclisation. If cyclisation were to occur first, then subsequent hydrolysis to the acid would be exactly similar to the hydrolysis of the preformed uracil ester, and therefore the hydrolysis times for these two systems would be very similar.

The difference in the overall reaction time for these two systems must therefore be due to a variation in the hydrolysis
Fig. 13 5-Carboxyuracils: Preparation from acyclic carbamate and uracil-5-carboxylic acid esters.

<table>
<thead>
<tr>
<th>1-substituent</th>
<th>Time for complete acyclic cyclisation-hydrolysis</th>
<th>Time for ester uracil hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>Reflux 1 min R. T. 10 min</td>
<td>R. T. 1 h</td>
</tr>
<tr>
<td>Me</td>
<td>Warm 2 min</td>
<td>R. T. 1 h</td>
</tr>
<tr>
<td>Ph</td>
<td>Warm 2 min</td>
<td>R. T. 1 h</td>
</tr>
<tr>
<td>Ph CH CH</td>
<td>Warm 2 min</td>
<td>Reflux 2 h</td>
</tr>
</tbody>
</table>

\[
\text{H} \quad \xrightarrow{\text{Reflux 1 min}} \quad \text{R. T. 10 min} \quad \xrightarrow{\text{R. T. 1 h}}
\]

\[
\text{Me} \quad \xrightarrow{\text{Warm 2 min}} \quad \text{R. T. 10 min} \quad \xrightarrow{\text{R. T. 1 h}}
\]

\[
\text{Ph} \quad \xrightarrow{\text{Warm 2 min}} \quad \text{R. T. 1 h} \quad \xrightarrow{\text{Reflux 2 h}}
\]

\[
\text{Ph CH CH} \quad \xrightarrow{\text{Warm 2 min}} \quad \text{Reflux 2 h}
\]
rates of the acyclic and uracil ester groups. The reaction mechanism shown in fig. (14) is a possible explanation for these differing hydrolysis rates.

Nucleophilic attack by a hydroxide ion on the central carbon atom of the ester group would lead to the formation of the intermediates A and B. This reversible step is followed by an irreversible step which drives the reaction to completion, with the liberation of an ethoxide ion. Any variation in the stability of the intermediates A and B, would therefore affect the ease with which the ethoxide ion was liberated, which in turn would be reflected in the overall rate of the hydrolysis reaction.

The intermediate A is likely to be the more stable intermediate due to the partial aromatic character of the uracil ring, which, acting as an electron sink, would deactivate the central carbon atom of the ester group. The ethoxide group in B would therefore be the more labile leaving group. Consequently the rate of decay of intermediate B would be greater than that of A. The irreversible breakdown of the intermediate is therefore likely to be the rate determinant step in this hydrolysis reaction.

Subsequent deprotonation of the amine group by the action of a hydroxide (or ethoxide) ion, followed by ring closure, with the elimination of an ethoxide ion, completes the hydrolysis-cyclisation sequence of the acyclic carbamate in alkaline solution.

b) Decarboxylation of uracil-5-carboxylic acids

The ease with which decarboxylation of the uracil carboxylic acids occurs, appears to vary directly with the degree of basicity of the decarboxylation medium. The minimum temperature found to be
effective in our experiments however was such as to restrict the selection of decarboxylating agents to those with a reasonably high boiling point. From the information contained in fig. 15, it may be seen that the efficiencies of the various decarboxylating agents varied thus:

Quinoline $< N$$\text{-dimethylaniline} < N$$\text{-ethylmorpholine}$ with the latter causing decarboxylation to occur at a temperature just below its own boiling point of 134°. $N$-Ethylmorpholine was therefore chosen as the main decarboxylating agent for this series of compounds.

c) Absorption Spectra (u.v.)

It can be seen from the data in fig. 16 that, in neutral solution, the uracils discussed in the chapter show u.v. absorption maxima in two main regions. One in the region $\lambda_{\text{max}}$ 287-259 nm and the other in the region $\lambda_{\text{max}}$ 237-213 nm. The ester uracils absorb at the top end of each range, $\lambda_{\text{max}}$ 287-277 nm and $\lambda_{\text{max}}$ 237-223 nm. The acid derivatives show absorption in the middle of each range at $\lambda_{\text{max}}$ 280-275 nm, and $\lambda_{\text{max}}$ 221-214 nm and the decarboxylated uracils absorb at the lower end of each range at $\lambda_{\text{max}}$ 272-259 nm and $\lambda_{\text{max}}$ 213-205 nm. In alkaline solution, only the 5-carboxyuracils are significantly affected, suffering downfield alkaline shifts to give absorption maxima similar to those for the decarboxylated derivatives, in the ranges $\lambda_{\text{max}}$ 274-260 nm and $\lambda_{\text{max}}$ 213-206 nm.

It may also be noticed that the nature of the $N_1$ substituent on the uracil ring causes variation in the position of the absorption maxima, such that a 1-alkyluracil absorbs at longer wavelength than a 1-aryluracil, which in turn absorbs at a longer wavelength than the $N_1$ unsubstituted uracils.
**Fig. 15** Decarboxylation temperatures required by 5-carboxy-uracil(208) and 5-carboxy-1-methyluracil(211).

<table>
<thead>
<tr>
<th>R</th>
<th>Heat solid</th>
<th>Heat solid + copper salt</th>
<th>Quinoline + copper</th>
<th>N,N-Dimethyl aniline</th>
<th>N-Ethyl morpholine</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>280°</td>
<td>275°</td>
<td>200-210°</td>
<td>190°</td>
<td>120-130°</td>
</tr>
<tr>
<td>Me</td>
<td>260°</td>
<td>-</td>
<td>210-220°</td>
<td>160-180°</td>
<td>120-130°</td>
</tr>
</tbody>
</table>

**Fig. 16** U.v. data for 1,5-substituted uracils and 1-substituted uracils (λ_max values quoted in nm).

<table>
<thead>
<tr>
<th>N₁ substituent</th>
<th>pH</th>
<th>COOEt</th>
<th>CO₂H</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td></td>
<td>277</td>
<td>271</td>
<td>259</td>
</tr>
<tr>
<td></td>
<td></td>
<td>224</td>
<td>217</td>
<td>205</td>
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The synthesis of 1-glycopyranosyluracils unsubstituted in the 5-position

The reaction sequence for the preparation of 1-substituted uracils was extended to cover the syntheses of 1-uracil nucleosides, by the reaction of ethyl N-(α-ethoxycarbonyl-β-ethoxyacryloyl)-carbamate (205) with the appropriate glycopyranosylamines and glycofuranosylamines. The glycosylamines involved in this section were D-xylopyranosylamine (224); D-glucopyranosylamine (225); L-rhamno-pyranosylamine (226); D-ribopyranosylamine (227); 3,5-0-isopropylidene-β-D-xylofuranosylamine (228); 2,3:5,6-di-0-isopropylidene-α-D-mannofuranosylamine (229); 2,3-0-isopropylidene-β-D-ribofuranosylamine (230); 2,3-0-isopropylidene-α-L-rhamnofuranosylamine (231) and 5,6-0-isopropylidene-β-D-glucofuranosylamine (232), the glycofuranosylamines being prepared as toluene- p-sulphonates, using a new synthesis developed by Cusack, Rugg and Shaw, which is discussed in more detail in the next chapter.

The glycopyranosylamines were easily prepared by dissolving the appropriate sugar in saturated methanolic ammonia at room temperature. The amines being obtained as crystals from the cooled solutions over several days.

D-Xylopyranosylamine (224) and ethyl N-(α-ethoxycarbonyl-β-ethoxyacryloyl)carbamate (205) in D.M.S.O. gave, on dilution with ether, ethyl N-(α-ethoxycarbonyl-β-D-xylopyranosylaminoacryloyl)carbamate (233) as a crystalline solid, m.p. 147-149. The mass spectrum of (233) supported the assigned structure, in that it showed a mass ion corresponding to that required for C$_{16}$H$_{11}$N$_{1}$O$_{4}$, together with subsidiary peaks indicating loss of ethanol, due to cyclisation.
being induced on the probe, and recognisable sugar and base fragments.

With sodium ethoxide in ethanol/D.M.S.O., cyclisation of the acyclic glycoside (233) occurred and neutralisation of the solution with Dowex 50W-X8 (H⁺) resin eventually gave 5-ethoxycarbonyl-1-β-D-xylopyranosyluracil (234) (m.p. 172-173).

The acyclic glycosylcarbamate (233) was easily converted into 5-carboxy-1-β-D-xylopyranosyluracil (235) by hydrolysis with aqueous sodium hydroxide solution. The acid separated as a crystalline solid (m.p. 188° decomp) upon neutralisation of the hydrolysis solution with Dowex 50W-X8 (H⁺) resin, which gave short needles, m.p. 192° from water (decomp).

Decarboxylation of the acid (235) was attempted by three methods:

a) A suspension of the acid in a xylene solution of p-toluene sulphonic acid was boiled under reflux for 2 h. The starting material was recovered unchanged.

b) A suspension of the acid in N,N-dimethylaniline was heated at 180° for 10 min. During this time carbon dioxide was evolved, however, extensive degradation of the solid rendered the subsequent purification of the uracil impossible.

c) A suspension of the acid in N-ethylmorpholine was heated at 135° for 10 min. Gaseous evolution occurred and 1-β-D-xylopyranosyluracil (236) was isolated as an amorphous solid, which gave needles, m.p. 220° from aqueous ethanol.

The mass spectrograph of the uracil (236) supported the assigned structure, showing a mass ion corresponding to the required structure, together with subsidiary peaks corresponding to base and sugar fragments. U.v. absorption peaks for the uracils prepared in this
chapter are to be found in fig. 17.

Ethyl N-(α-ethoxycarbonyl-β-D-glucopyranosylaminoacryloyl)-carbamate(238), m.p. 153°, was obtained as a crystalline solid from an ethanolic solution containing the ethyl carbamate(205) and D-glucopyranosylamine(225). Cyclisation of this acyclic carbamate (237) with alcoholic sodium ethoxide solution gave 5-ethoxycarbonyl-1-β-D-glucopyranosyluracil(238) as a crystalline solid (m.p. 244°).

The acyclic carbamate(237), when treated with aqueous sodium hydroxide solution, hydrolysed and cyclised to give 5-carboxy-1-β-D-glucopyranosyl— uracil(239) which was obtained as a solid, m.p. 220° (decomp), by evaporation to dryness in vacuo of the neutralised aqueous solution. A recrystallised sample of the acid (239) readily effervesced with sodium bicarbonate, and easily decarboxylated during mass spectroscopic analysis. The mass spectrum showed a large mass ion peak corresponding to that required for (239) together with peaks corresponding to the decarboxylated uracil (240), and the sugar fragment.

Several attempts were made to prepare the glucopyranosyluracil (240) by decarboxylation of the glucopyranosyl acid(239) and these are detailed below:

a) Heating the acid derivative in D.M.S.O. under reflux, the progress of the reaction being followed both visually and spectrophotometrically, making use of the characteristic u.v. absorption shift in neutral solution from λmax 268 and 213 nm to λmax 264 and 205 nm. No evidence was found to suggest that decarboxylation had taken place although the final product was too badly degraded to obtain as a pure sample.

b) Heating a small sample of the acid uracil derivative in N-N-di-
methylaniline at 180 for 10 min, resulted in gaseous evolution. However, the subsequent work-up of the degraded residue proved to be rather difficult.

c) A mixture of the solution of the acid in quinoline and copper powder was heated and evolution of carbon dioxide gas, detectable with limewater, commenced at 180°. However, extensive degradation occurred which rendered the final work-up of the product very difficult.

d) A suspension of the glucopyranosyl acid in N-ethylmorpholine when heated at 130° for some 15 min, gave the decarboxylated glycosyluracil as an amorphous powder. 1-ß-D-Glucopyranosyluracil(240) was obtained as needles (m.p. 238°) from aqueous ethanol. During this last decarboxylation attempt, u.v. analysis showed a rapid and progressive shift in the absorption spectrum from λmax 268 and 213 nm, characteristic of the 5-carboxyuracil, to λmax 264 and 205 nm, characteristic of the 5-unsubstituted uracil.

A solution of L-rhamnopyranosylamine (226) and ethyl N-(α-ethoxy carbonyl-ß-ethoxyacryloyl)carbamate (205) in D.M.S.O. which had been allowed to stand overnight at room temperature gave, on dilution with alcohol and ether, ethyl N-(α-ethoxycarbonyl-ß-L-rhamnopyranosylaminoacryloyl)carbamate (241) as a white powder, m.p. 178-180° (decomp), in reasonably high yield (69%). The acyclic carbamate (241) was converted directly into the uracil-carboxylic acid (242) by reaction with aqueous sodium hydroxide solution. Neutralisation with sulphuric acid and dilution with alcohol, precipitated sodium sulphate in near quantitative yield. Cooling of the mother liquors gave 5-carboxy-1-α-L-rhamnopyranosyluracil (242) as a gel, which slowly crystallised over three to four days and effervesced with sodium bicarbonate solution. The u.v. spectrum of (242) was again found
to be characteristic of the 5-carboxyuracil ring system, showing absorption maxima at \( \lambda_{\text{max}} \) 269 and 213 nm in neutral solution.

Decarboxylation of the rhamnopyranosyluracil acid (242) was achieved by heating a suspension of the acid in N-ethylmorpholine to 130-135\(^\circ\). Carbon dioxide evolution was rapid and completed within 1h. During the reaction the u.v. absorption spectrum changed progressively from \( \lambda_{\text{max}} \) 269 and 213 nm to that characteristic of the unsubstituted uracil with \( \lambda_{\text{max}} \) 264 and 205 nm in neutral solution. Recovery and purification of the suspension gave 1-\(\alpha\)-L-rhamnopyranosyluracil (243) as platelets, m.p. 258(decomp.).

A similar reaction scheme was initiated for the preparation of 1-\(\beta\)-D-ribopyranosyluracil (247), however, lack of time prevented completion of this scheme.

5-Ethoxycarbonyl-1-\(\beta\)-D-ribopyranosyluracil (245) was prepared by the reaction of D-ribopyranosylamine (227) and ethyl N-(\(\alpha\)-ethoxycarbonyl-\(\beta\)-ethoxyacryloyl)carbamate (205) in D.M.S.O. Cyclisation of the acyclic compound ethyl N-(\(\alpha\)-ethoxycarbonyl-\(\beta\)-D-ribopyranosylaminoacryloyl)carbamate (244) to the ribopyranosyluracil (245) was performed in situ by the addition of sodium ethoxide. Dilution with alcohol gave a precipitate of the sodium salt of the uracil (246), which was dissolved in water and neutralised with Dowex 50W-X8 (H\(^+\)) resin. The resulting solution at 0\(^\circ\) gave 5-ethoxycarbonyl-1-\(\beta\)-D-ribopyranosyluracil (245) as fine needles over several days, m.p. 188(decomp). The u.v. absorption spectrum of (246) was found to be characteristic of 5-ethoxycarbonyl uracils, with \( \lambda_{\text{max}} \) 274 and 220 nm in neutral solution.

From the data contained in fig. 17, it can be seen that the u.v. absorption peaks for the above glycopyranosyluracils fall within
bands similar to those for the aglycone derivatives but at generally lower wavelengths, one band being at $\lambda_{\text{max}}$ 220-204 nm and the other at $\lambda_{\text{max}}$ 274-263 nm, compared to $\lambda_{\text{max}}$ 237-212 nm and 285-274 nm respectively for the aglycone derivatives. The same characteristic shifts are however in evidence.

The absorption maxima in neutral solution of the ester derivatives are found at highest wavelengths, $\lambda_{\text{max}}$ 274-272 nm and $\lambda_{\text{max}}$ 220-214 nm, the acid uracils absorb in the ranges $\lambda_{\text{max}}$ 269-268 nm and $\lambda_{\text{max}}$ 214-213 nm and the decarboxylated uracils absorb in the ranges $\lambda_{\text{max}}$ 265-263 nm and $\lambda_{\text{max}}$ 206-205 nm.

In alkaline solution the absorption maxima of the ester uracils and the decarboxylated uracils are largely unaffected, whereas the carboxyuracils suffer a pronounced downfield alkali shift to give absorption maxima in the regions corresponding to the decarboxylated uracils, at $\lambda_{\text{max}}$ 265-263 nm and $\lambda_{\text{max}}$ 206-205 nm.
Fig. 17 U.v. data for 1-glycopyranosyluracils (λ<sub>max</sub> values quoted in nm).

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CHAPTER SIX

The synthesis of 1-glycofuranosyluracils unsubstituted in the 5-position

Interesting though the synthesis of glycopyranosyl uracils might be, it is with the glycofuranosyl derivatives that the possible benefits of such a reaction scheme might lie. For it is the glycofuranoside derivative which offers the possibility of incorporation into natural systems and hence the possibility of possessing chemotherapeutic activity.

As has been shown above, glycopyranosyluracils may be prepared, with reasonable ease, by the reaction between ethyl N-(α-ethoxy carbonyl-β-ethoxyacryloyl)carbamate(205) and a suitable glycopyranosylamine, with hydrolysis, cyclisation and decarboxylation of the linear product.

The adaptation of this method to the synthesis of glycofuranosyl derivatives is dependent upon the availability of suitable glycofuranosylamines. One route to these reagents was developed by Baddiley, Buchanan, Hodges and Prescott which gave the ribofuranosylamine tri-O-benzoate(157) by reduction of the corresponding tribenzoylribofuranosylazide(156), which was prepared in turn by condensation of the 2,3,5-tribenzoylribofuranosylchloride with sodium azide. This route is tedious and suffers mainly from the requirement that the ribofuranosylamine has generally to be made in situ and used immediately, otherwise O→N migration of the 2-O-benzoyl group occurs.

A simple synthesis of a stable ribofuranosylamine derivative which can be used for direct preparation of many types of nucleosides was recorded by Cusack and Shaw. Condensation of D-ribose with
saturated methanolic ammonia at 0° readily gave the easily
1\textsuperscript{8} crystallised D-ribopyranosylamine(227) in 90% yield. Evidence for
the pyranose nature of this material came from its condensation
with ethyl N- (\(\alpha\)-cyano-\(\beta\)-ethoxyacryloyl)carbamate(124) to afford the
ribopyranosyluracil(248) which was shown to absorb 2 mole equivalent
of periodate thereby giving 1 mole equivalent of formic acid. In
contrast the furanose derivative(249), subsequently prepared by a
similar condensation of the benzoylated amine(157) with the carbamate
(124) and hydrolysis of the derived nucleoside, absorbed 1 mole
equivalent of periodate with no acid production.

The ribopyranosylamine(227), when stirred with 2,2-dimethoxy-
propane, acetone, and toluene-\(p\)-sulphonic acid gave the crystalline
2,3-\(\text{O}\)-isopropylidene-\(\beta\)-D-ribofuranosylamine as a toluene-\(p\)-sulph-
onate(230).

The structure of the compound followed from elemental analysis,
mild acidic hydrolysis to give acetone, i.r. characteristic doublet
at 1380 cm\(^{-1}\), n.m.r. spectra, and chemical reactions. A solution of
the compound in chloroform was found to exist essentially as the
pure \(\beta\)-anomer. In D.M.S.O., however, n.m.r. signals appeared for
both \(\alpha\)-and \(\beta\)-anomers. An aqueous solution was found to mutarotate
rapidly whereas a solution in alcohol mutarotated more slowly.

In preliminary experiments the reaction of the isopropylidene-
ribofuranosylamine(230) with the ethoxycarbamate(124) in the
presence of sodium methoxide in methanol gave the 5-cyanoisoprop-
ylideneuridine(250) which, with aqueous acid, gave the corresponding
5-cyanouridine(249). This compound was identical with an authentic
sample prepared from the benzoylated amine(157) and the carbamate
(124). In later experiments however, the presence was noted of a
second u.v. absorbing material similar to the \(\beta\)-derivative. This
was readily separated and proved to be the \( \alpha \)-form (251).

The generality of the reaction leading to the facile formation of glycofuranosylamine toluene-\( \beta \)-sulphonates was investigated, in particular to see if it could be extended to the preparation of glycofuranosylamines, from pyranosylamines whose structures might be expected to favour formation of furanose configurations. It was found that the reaction may be extended to include synthesis of glycofuranosylamines derived from D-xylose, D-mannose, and L-rhamnose.

Reaction of D-xylopyranosylamine (224), D-mannopyranosylamine (252) or L-rhamnopyranosylamine (226), all readily available from the sugar and methanolic ammonia, with acetone, 2,2-dimethoxypropane and toluene-\( \beta \)-sulphonic acid gave in excellent yield, 3,5-O-isopropylidene-\( \beta \)-D-xylofuranosylamine toluene-\( \beta \)-sulphonate (228) which crystallised directly from the reaction mixture after 5 min, and had m.p. 121\( ^\circ \text{C} \) (decomp). 2,3:5,6-di-O-isopropylidene-\( \alpha \)-D-mannofuranosylamine toluene-\( \beta \)-sulphonate (229), which separated, after addition of ether, as a crystalline solid, m.p. 132-134\( ^\circ \text{C} \) (decomp), and 2,3-O-isopropylidene-\( \alpha \)-L-rhamnofuranosylamine toluene-\( \beta \)-sulphonate (231) which also separated as a crystalline solid, m.p. 143\( ^\circ \text{C} \) (decomp), after addition of ether.

The structural assignments were confirmed by elemental analysis, formation of acetone (identified as its 2,4-dinitrophenylhydrazone) on acid hydrolysis, and reaction with ethyl N-(\( \alpha \)-cyano-\( \beta \)-ethoxyacryloyl)carbamate (124) in the case of mannofuranosylamine, to give the corresponding uracil derivative (225), m.p. 85\( ^\circ \text{C} \), or in the case of xylo- and rhamnofuranosylamines with compound (67) to afford the uracils (253), m.p. 220-222\( ^\circ \text{C} \) (decomp) and (254) m.p. 272\( ^\circ \text{C} \) (decomp) respectively.
The isopropylidene groups in each of the compounds (253), (254) and (255) could be removed by heating with dilute aqueous acid to give the corresponding uracils (256), m.p. 197° (decomp); (257), m.p. 142° (decomp) and (258), m.p. 218° (decomp). The structures of the latter uracils were further confirmed by elemental analyses and mass and u.v. absorption spectra (characteristic of 1-substituted 5-cyanouracils). In addition, in each case the furanoses were compared with, and shown to be different from, the corresponding pyranosyluracils obtained in high yield by similar condensations of the acryloyl derivatives (124) and (67) with the appropriate pyranosylamines.

In all cases a predominant trans-arrangement is assumed to exist between the sugar 2'-hydroxy group and the uracil ring leading to α-configurations for the rhamnose and mannose derivatives and a β-configuration for the xylose derivatives. Uracil formation in each case must proceed through initial formation of the linear carbamate derivative, an examination of molecular models suggests that cyclisation of the form likely to give a trans-arrangement between the 2'-hydroxy group and the uracil ring should occur more readily than with the corresponding form likely to give a cis arrangement. In the latter forms, the -NH group is sterically hindered by the 2'-O-substituent.

The above glycofuranosylamine toluene-π-sulphonates were used in an attempt to synthesise glycofuranosyluracils analogous to the pyranosyl derivatives already mentioned. As the ribofuranosyluracil is a readily available natural product, uridine(158), it was decided to concentrate on this compound, as unambiguous proof of structure would be available, by comparison with an authentic sample.
The ethoxyacrylamide(205) and 2,3-O-isopropylidene-β-D-ribofuranosylamine toluene-β-sulphonate(230) gave, presumably via the acyclic riboside ethyl N-(α-ethoxycarbonyl-2,3-O-isopropylidene-β-D-ribofuranosylaminoacryloyl)carbamate(259), the ester uracil, 5-ethoxycarbonyl-1-(2',3'-O-isopropylidene-β-D-ribofuranosyl)uracil(260).

This reaction was performed in a number of solvents viz., methanol, ethanol, t-butanol, ethyl acetate and cyano-methane, and a number of basic reagents were used to release the ribofuranosylamine and to initiate ring closure of the acyclic intermediate(259), these being, sodium methoxide, sodium ethoxide, potassium t-butoxide and triethylamine. No ideal system was found as each had disadvantages. Methanol and sodium methoxide gave a reasonable yield of the acyclic carbamate (259), however, there was a tendency for hydrolysis of the ethoxycarbonylacrylamide(205) to occur, yielding the acyl carbamate(204). Hydrolysis did not occur with ethanol and sodium ethoxide nor with the t-butanol system, however, solubility problems tended to drastically reduce yields. Ethyl acetate and cyanomethane possessed no obvious advantages and triethylamine, though more convenient to use was found to be too weak a base, especially for the second stage of the reaction, to give, by base induced cyclisation, the ethoxycarbonyl-uracil(260).

The system found to be most convenient was sodium ethoxide and methanol. 2,3-O-Isopropylideneribofuranosylamine was liberated from its toluene-β-sulphonate salt(230) by the addition of 1 mole equivalent of sodium ethoxide to a methanolic solution of the salt (230). Reaction between the free glycofuransylamine and the ethoxycrylicarbamate(205) gave the acyclic intermediate(259). A second mole equivalent of sodium ethoxide was used to induce cyclisation to the uracil(260), which was obtained as the sodium
sald of the uracil as a golden coloured oil. Neutralisation of an aqueous solution of this oil gave crystals of 5-ethoxycarbonyl-1-(2',3'-O-isopropylideneribofuranosyl)uracil(260) in 67% yield, the reaction being followed by u.v. analysis, the ester (260) having absorption peaks at $\lambda_{\text{max}} 274$ and 219 nm.

Hydrolysis of the ester (260) to the carboxyuracil (261) was achieved only by maintaining an alkaline solution of the ester (260) above 70° for 2 h. Neutralisation and evaporation gave a solid white foam, m.p. 186-192°, from which 5-carboxy-1-β-D-(2',3'-O-isopropylidene-β-D-ribofuranosyl)uracil (261) was obtained as a crystalline solid, m.p. 190-193°. Absorption peaks (u.v.) were found at $\lambda_{\text{max}} 272$ and 216 nm.

If the initial reaction mixture of the ethoxycrylamide (205) and the ribofuranosylamine salt (230), containing the acyclic intermediate (259), was treated with aqueous alkali instead of sodium ethoxide, the 5-carboxyuracil (261) was formed directly. Isolation of the carboxyuracil (261) by neutralisation, evaporation and recrystallisation gave a product identical to the sample obtained by hydrolysis of the 5-ethoxycarbonyluracil (260).

Hydrolysis of the ester with barium hydroxide was attempted, however, an immediate precipitate was obtained, presumably of the barium salt of the ester. The addition of barium hydroxide to the initial reaction mixture containing the acyclic intermediate (259), gave an immediate precipitate, again of the uracil ester salt, in high yield. However, this modification to the reaction scheme was not as useful, in terms of purification of the ethoxycarbonyluracil, as it first appeared, as neutralisation and purification of this salt were found to be rather difficult.

Decarboxylation of the carboxylic acid (261) followed the lines
already laid down for the 1-glycopyranosyluracils. Carbon dioxide was evolved when the compound was heated to 260°, however, the sample was extensively degraded at this temperature.

Heating the carboxyuracil(261) in N-ethylmorpholine proved to be a readily applicable method for decarboxylation, a temperature of 130-135° for 1h, being sufficient to ensure complete decarboxylation. The decarboxylation reaction was followed by u.v. analysis, the absorption peaks at $\lambda_{\text{max}}$ 272 and 216 nm of the acid(261) moving downfield to $\lambda_{\text{max}}$ 267 and 214 nm respectively upon decarboxylation, to give 1-(2',3'-O-isopropylidene-$\beta$-D-ribofuranosyl)uracil(262). The isopropylideneuracil nucleoside(262) after recovery from the N-ethylmorpholine suspension was readily hydrolysed, by aqueous acetic acid, to give uridine(158). Evaporation of this solution gave a solid foam, an alcoholic solution of which was found to have a strong u.v. absorption peak, corresponding exactly with an authentic sample of uridine, at $\lambda_{\text{max}}$ 262nm. Uridine(158) was precipitated from the alcoholic solution by the addition of ether, and was found to be identical (m.spec.,u.v.,i.r.,m.p., and t.l.c.) with an authentic sample.

By a similar reaction sequence to the one described above for the synthesis of uridine, the syntheses of other uracil nucleosides was attempted. The furanosylamine toluene-$\beta$-sulphonate salts involved were those of xylofuranosylamine(228), mannofuranosylamine(229), rhamnofuranosylamine(231) and glucofuranosylamine(232). Limited time however, prevented more than a token attempt being made with the rhamno- and glucofuranosylamines.

The free glycosylamine, 3,5-O-isopropylidene-$\beta$-D-xylofuranosylamine was released from its toluene-$\beta$-sulphonate salt(228) by the
addition of 1 mole equivalent of sodium ethoxide. After warming with ethyl N-(α-ethoxycarbonyl-β-ethoxyacryloyl)carbamate (205) and a second mole equivalent of sodium ethoxide, a sticky white precipitate of the sodium salt of the xylofuranosyluracil ester (263) was obtained on dilution with ether. Neutralisation and evaporation of an aqueous solution of the precipitate gave a brown coloured solid foam (5.8 g, 40 %), which gave 5-ethoxycarbonyl-1-(3’,5’-0-isopropylidene-β-D-xylofuranosyl)–uracil (263) as short needles, m.p. 190° (decomp), from water, which absorbed in the u.v. at λmax 273 and 220 nm.

Hydrolysis of the ester uracil (263) was achieved by boiling an alkaline solution under reflux for 2 h, which gave on neutralisation and evaporation to dryness, 5-carboxy-1-(3’,5’-0-isopropylidene-β-D-xylofuranosyl)uracil (264), as a solid white foam, m.p. 140° (decomp), which effervesced with aqueous sodium bicarbonate solution (u.v. λmax 270 and 215 nm).

The carboxyuracil (264) was decarboxylated to the isopropylidene nucleoside (265) by heating in N-ethylmorpholine. The suspension was maintained at 130° for 45 min, during which time, the suspension softened, evolved carbon dioxide and resolidified to give a hard amorphous solid. The suspension was heated in fresh N-ethylmorpholine for a further 30 min at 130° until all stickiness had gone from the suspension. The solid was recovered and ether washed. 1-(3’,5’-0-Isopropylidene-β-D-xylofuranosyl)uracil (265) gave crystals, m.p. 248°, with u.v. absorption maxima at λmax 265 and 214 nm.

The isopropylidene derivative (265) was readily hydrolysed by aqueous acetic acid to the uracil nucleoside, 1-xylofuranosyluracil (266), which gave needles from ethanol, m.p. 263° (decomp) and showed u.v. absorption at λmax 261 nm.

The mannofuranosyl nucleoside (270) was prepared by an
(261)
exactly similar reaction sequence, in that 2,3:5,6-di-O-isopropylidene-α-D-mannofuranosylamine, liberated from its toluene-p-sulphonate salt(229) by sodium ethoxide, gave with the ethoxycarbonylacrylamide (205) the acyclic glycosylcarbamate, ethyl N-(α-ethoxycarbonyl-β-2',3':5',6'-di-O-isopropylidene-α-D-mannofuranosylaminoacryloyl) carbamate(267). (267) with a second mole equivalent of the base rapidly cyclised to the glycosyluracil(268), which was isolated by evaporation, dissolution in water and neutralisation with mineral acid, as a precipitate. Purification of this precipitate gave 5-ethoxycarbonyl-1-(2',3':5',6'-di-O-isopropylidene-α-D-mannofuranosyl)uracil(268), m.p. 210-214° (decomp), in 64% yield. A sample of (268) was found to absorb in the u.v. at λmax 278 and 222 nm.

The ester uracil(268) was hydrolysed to the 5-carboxy derivative (269) by heating in alkaline solution for some 90 min. A solid foam was obtained from the neutralised solution which gave 5-carboxy-1-(2',3':5',6'-di-O-isopropylidene-α-D-mannofuranosyl)uracil(269), m.p. 242° (decomp), on recrystallisation, in 62% yield. U.v. absorption analysis showed absorption peaks at λmax 265 and 210 nm.

Decarboxylation of (269) by heating as a suspension in N-ethyl-morpholine for 1h at 135 gave 1-(2',3':5',6'-di-O-isopropylidene-α-D-mannofuranosyl)uracil(270) as a brown amorphous suspension, which gave plates, m.p. 250° (decomp), from ethyl acetate-ethanol.

The isopropylidene nucleoside (270) was readily hydrolysed to the uracil nucleoside(271) by heating in aqueous acetic acid for some 3h. Evaporation gave a solid foam, from which 1-α-D-mannofuranosyluracil(271) was obtained as a crystalline material, m.p. 253° (decomp).

By exactly analogous reactions to those above, the isopropylidenerhamno- and isopropylidenegluco- furanosyluracil esters(272), m.p. 186° (decomp) and (273), m.p. 202-214° (decomp) respectively were
prepared. Unfortunately lack of time prevented further investigation of these compounds.

From the u.v. data given in fig. 18, it may be seen that the glycofuranosyluracils described above also absorb in the same general regions as the alkyl(aryl)- and glycopyranosyl- uracils described in chapters 4 and 5. The same two general absorption regions are observed at $\lambda_{\text{max}}$ 278-261 nm and $\lambda_{\text{max}}$ 222-210 nm, again with the ester derivative absorbing at the upper end of each band, $\lambda_{\text{max}}$ 278-274 nm and $\lambda_{\text{max}}$ 222-219 nm; the carboxy derivative absorbing in the middle regions, $\lambda_{\text{max}}$ 272-270 nm and $\lambda_{\text{max}}$ 216-212 nm, and the isopropylideneuracil nucleosides absorbing at $\lambda_{\text{max}}$ 267-265 nm and $\lambda_{\text{max}}$ 214-210 nm. The hydrolysed 'free' glycofuranosyluracils absorbed at the bottom end of the upper region, $\lambda_{\text{max}}$ 262-261 nm with no second absorption peak being observed within the lower region.

It would appear from the foregoing results that formation of 5-ethoxycarbonyluracils by reaction of the ethoxycarbonylacrylamide (205) with suitable amines, followed by hydrolysis and decarboxylation does offer a useful route to 1-substituted uracils. The route is of particular use in the synthesis of 1-glycosyluracils, especially when the amine used is an isopropylideneglycofuranosylamine, thereby providing a valuable route to uracil nucleosides.
Fig. 18 U.v. data for 1-glycofuranosyluracils in neutral solution ($\lambda_{\text{max}}$ values quoted in nm).

<table>
<thead>
<tr>
<th>N$_1$ glycosyl substituent</th>
<th>Isopropylidene derivative</th>
<th>Free glycosyl uracil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C$_6$ substituent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>COOEt</td>
<td>COOH</td>
</tr>
<tr>
<td>D-Ribose</td>
<td>274</td>
<td>272</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>272</td>
<td>270</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>278</td>
<td>271</td>
</tr>
</tbody>
</table>
CHAPTER SEVEN

The synthesis of 1-alkyl(aryl)-3-methyluracils unsubstituted in the 5-position.

In order to prepare 3-methyluracils of the type (274) similar to the aglycone derivatives previously described, a new reagent was prepared, ethyl N-(α-ethoxycarbonyl-β-ethoxy-N-methylacryloyl)-carbamate (276).

![Chemical structure](image)

Three routes to this compound were attempted and they are illustrated schematically in fig. 19.

**Route a)** involved a reaction sequence exactly similar to that of the ethoxycarbonyl reagent (205) in which ethylcarbamate was replaced by ethyl N-methylcarbamate, which with ethyl hydrogen malonate gave the N-methyl analogue (275) of the ethoxycarbonyl-carbamate (204). Ethyl N-methyl-N-ethoxycarbonylacetylcarbamate (275) with triethylorthoformate in acetic anhydride gave the required N-methyl reagent, ethyl N-(α-ethoxycarbonyl-β-ethoxy-N-methylacryloyl)carbamate (276).

**Route b)** involved N-methylation of the ethoxycarbonylcarbamate (204) to give ethyl N-methyl-N-ethoxycarbonylacetylcarbamate (275) which was then treated with triethylorthoformate as above.

**Route c)** involved direct N-methylation of ethyl N-(α-ethoxycarbonyl-β-ethoxyacryloyl)carbamate (205) to give the N-methyl derivative (276).
All three routes were investigated and the following results were obtained.

**Route a)** Ethyl hydrogen malonate and acetic anhydride were warmed together to allow formation of the mixed anhydride(277). Ethyl N-methylcarbamate, prepared by the reaction between methylamine and ethyl chloroformate, when added to the reaction mixture gave, after heating for 3 h, a solution from which, by evaporation, and vacuum distillation, two main fractions were isolated. The first fraction was found to contain mostly N-methylacetylcarbamate(278), formed by an alternative reaction of the mixed anhydride(277) and ethyl N-methylcarbamate. The second fraction gave, in 59% yield, the required product, ethyl N-methyl-N-ethoxycarbonylacetylcarbamate (275).

Triethyl orthoformate and acetic anhydride when warmed together gave a solution containing the intermediate(279), the presence of which was necessary, if the subsequent reaction of the orthoformate and ethyl N-methyl-N-ethoxycarbonylacetylcarbamate(275) was to give a reasonable yield of ethyl N-(α-ethoxycarbonyl-β-ethoxy-N-methylacryloyl)carbamate(276).

Numerous experiments were performed to optimise the yield of this reaction, and it was found that a temperature of 85°C for 3 h gave the best yield. Evaporation and vacuum distillation of the reaction mixture gave two fractions, the second of which was found to be the required N-methylcarbamate(276) by elemental analysis, mass spectrometry and reaction with simple amines to give 1-substituted-5-ethoxycarbonyl-3-methyluracils of the type (274).

**Route b)** Treatment of an ethereal suspension of ethyl N-ethoxycarbonylacetylcarbamate(204), with diazomethane gave a vigorous
reaction which slowly produced a solution from which, by evaporation and vacuum distillation two major fractions were obtained. The second of these fractions was found to be identical to the sample of ethyl N-methylacetylcarbamate (275) obtained by route a). The reaction of this sample with triethyl orthoformate and acetic anhydride gave a product identical to ethyl N-(α-ethoxycarbonyl-β-ethoxy-N-methylacryloyl)carbamate (276), obtained by route a).

Route c) N-Methylation of ethyl N-(α-ethoxycarbonyl-β-ethoxyacryloyl)carbamate (205) to give the N-methyl derivative (276) directly, was attempted in a number of different ways.

  c(i) Using methyl iodide: A solution of the ethyl carbamate (205) in acetone was treated with potassium carbonate and methyl iodide. Dilution with ether gave a precipitate of potassium iodide and the starting material (205). The mother liquors, when evaporated gave an oily solid which failed to react with a sample of phenylethylamine. Other evidence (mass spectrometry and t.l.c.) indicated the presence of a small quantity of the required compound in the final product, which was separated by vacuum distillation, a small sample of the N-methylcarbamate (276) (5% yield) being isolated.

  c(ii) Using diazomethane: An ethereal suspension of the ethylcarbamate (205) when treated with excess diazomethane gave a vigorous reaction, and the suspension dissolved. The excess diazomethane was destroyed and the solution was evaporated under vacuum to give an oil which failed to react with any primary amine. From mass spectrometry data it was found that the oil had a mass of 287 with a large secondary peak at 273. The loss of 14 could correspond to the loss -CH₂-. It was suspected that the required methylation initially occurred, followed by the reaction of a second mole of diazomethane.
across the unsaturated bond to give the N-methyl derivative (280).

The calculated mass for derivative (280) \((C_{12}H_{18}NO_2)\) corresponded exactly to the peak found at 287.

c(iii) Using dimethyl sulphate: A sample of the ethylcarbamate (205) was refluxed with dimethyl sulphate for 2 h. Evaporation and recrystallisation from ethyl acetate gave back the starting material unchanged.

c(iv) Using dimethyl sulphate and the sodium salt of (205): The above method was modified to the extent of converting the ethylcarbamate (205) into its sodium salt by the addition of ethanolic sodium ethoxide, prior to the addition of dimethyl sulphate. The final solution after several hours at 0° gave a near quantitative precipitate of sodium methyl sulphate, and the mother liquors on evaporation gave an oil, rich in the required product, which was purified by vacuum distillation. This proved to be a most useful route to the N-methylcarbamate (276), preferred, even above the direct method involving reaction of ethyl N-methylcarbamate outlined in route a), on the grounds of both convenience (as all materials prior to methylation are solids and therefore easily purified) and overall yield.

c (v) Using dimethyl sulphate and the silver salt of (205): A further modification of the above scheme involved isolation of the silver salt of the ethylcarbamate (205) as a solid, by the reaction of a solution of the sodium salt of (205) with silver nitrate. The precipitate obtained was found to be extremely light sensitive, and so most unsuitable for subsequent reaction with dimethyl sulphate.

Several derivatives of ethyl N-(\(\alpha\)-ethoxycarbonyl-\(\beta\)-ethoxy-N-methylacryloyl)carbamate (276) were prepared by direct reaction of
with phenylethylamine, ammonia and methylamine to give acyclic products of the type (281), which upon cyclisation gave the appropriate 1-substituted 5-ethoxycarbonyl- or 5-carboxy-3-methyl-
uracil.

An ethanolic solution of the N-methylcarbamate (276), when treated with phenylethylamine gave, on dilution with water the acyclic carbamate (282) as an oil, which was collected. Dissolution of the oil in aqueous alkali caused cyclisation and hydrolysis of (282) to occur, and neutralisation of this solution with mineral acid gave, 5-carboxy-1-phenylethyl-3-methyluracil (284) as a precipitate (m.p. 248), which gave needles from water and effervesced with aqueous sodium bicarbonate solution. Mass spectrometry indicated a molecular weight of 274 which corresponded exactly to the mass required for (284) \((C_{19}H_{26}N_2O_4)\), with a secondary peak at mass number 230 which corresponded to loss of carbon dioxide, due to decarboxylation of the uracil (284) on the probe. If the initial reaction was performed in cooled ether then the vigorous exothermic reaction between (276) and phenylethylamine gave the acyclic carbamate ethyl N-(\(\alpha\)-ethoxycarbonyl-\(\beta\)-phenylethlamino-N-methylacryloyl)carbamate (282) as a white precipitate, m.p. 94°. This on heating, cyclised to give 5-ethoxycarbonyl-1-phenylethyl-3-methyluracil (283), m.p. 110°, with loss of ethanol.

The structure of (283) was confirmed by elemental analysis, mass spectrometry and by comparison with a sample prepared by N-methylation of 5-ethoxycarbonyl-1-phenylethyluracil (220) as detailed below.

A suspension of the uracil (220) in ether was treated with ethanolic diazomethane until all solid had dissolved and
apparent reaction ceased. Excess diazomethane was destroyed and the solution on evaporation gave an oil, soluble in ether, which was trituated under water giving an amorphous grey solid, no longer soluble in ether, which in turn gave in low yield from ethyl acetate, needles (m.p. 110°) of 5-ethoxycarbonyl-1-phenylethyl-3-methyluracil (283) identical (u.v. m.s., t.l.c., m.p., mixed m.p.) with the sample prepared above.

The acyclic carbamate ethyl N-(α-ethoxycarbonyl-β-amino-N-methylacyrloyl)carbamate (285) was prepared by passing ammonia gas into an ethanolic solution of the N-methylcarbamate (276), until a saturated solution was obtained. Evaporation gave an oil containing the acyclic carbamate (285). Treatment of this oil with aqueous alkali gave, on neutralisation, a precipitate of 5-carboxy-3-methyluracil (286), m.p. 220°, which effervesced readily with aqueous sodium bicarbonate.

Treatment of the oil with ethanolic sodium ethoxide followed by neutralisation with mineral acid gave 5-ethoxycarbonyl-3-methyluracil (287) as a precipitate which gave plates from ethyl acetate, m.p. 170°.

The methylamino derivative was prepared by the reaction between methylamine and the N-methylcarbamate (276). Evaporation of the reaction mixture gave the acyclic carbamate, ethyl N-(α-ethoxy-β-methylaminoacryloyl)carbamate (288) as an oil, which only crystallised at temperatures below -30°. Alkaline hydrolysis of (288) followed by neutralisation with mineral acid, gave a white precipitate of 5-carboxy-1,3-dimethyluracil (289) which gave plates from water, m.p. 212°.
All three 5-carboxy-3-methyluracils, (284), (286), and (289) decarboxylated readily on heating above 200 °C in air, or to 130 °C in N-ethylmorpholine, to yield the corresponding 3-methyluracils, 1-phenylethyl-3-methyluracil (290a), m.p. 253 °C; 3-methyluracil (290b), m.p. 234 °C; 1,3-dimethyluracil (290c), m.p. 217 °C, respectively.

The reaction sequence described above would appear to offer a useful route to 1,3-disubstituted uracils, with the option of C₅ substitution being readily available, via the 5-carboxy compound, or by electrophilic substitution of the C₅ unsubstituted uracil.

The preparation of N₅-substituted uracils via the N-alkylcarbamate reagent, analogous to (276), obtained by direct alkylation (or possible arylation) of the ethoxycarbonylcarbamate (205), by reaction of the alkyl sulphate with the sodium salt of (205), would appear to offer a simpler route than direct N₅ alkylation of the preformed uracil.

Of the two general methods used to prepare the N-alkylcarbamate reagent (of the type (276)), direct alkylation of (205) was preferred both in terms of overall yield and utility. The ethoxycarbonylcarbamate (205) could possibly be used to prepare a variety of N-alkylcarbamate reagents, and thereby facilitate the preparation of uracils with different N₅ substituents. The reaction between an N-alkylcarbamate (analogous to ethyl N-methylcarbamate (275)) with triethyl orthoformate to give the N-alkylcarbamate reagent of the type (276), however, would require prior preparation of a different N-alkylcarbamate corresponding to each required N₅ substituent in the uracil ring.
CHAPTER EIGHT

The synthesis of other substituted uracils

A number of the reagents used in the preparation, or attempted preparation, of the substituted pyrazolo[3,4-d]pyrimidine ring system, found elsewhere in this thesis, were utilised to prepare substituted uracils, by reaction of the reagent, with primary amines. The reagents investigated were, α-cyano-β-ethoxy-N-formylacrylamide (291); α-cyano-β-ethoxy-N-acetylacrylamide (292); 5-cyano-2-ethylthio-4-oxo-1,3-thiazine (72) and ethyl N-(α-cyano-β-ethoxy-N-methylacryloyl)carbamate (67).

1) Reactions of the N-formylacrylamide (291)

The reactions between the N-formylacrylamide (291) and aniline, methylamine and cyclohexylamine were investigated. The route to the uracil ring structure required the initial formation of an acyclic derivative (293), in an exactly analogous manner to that described in the preceding chapters, for the 5-ethoxy-carbonyluracils. Cyclisation to the 2-deoxyuracil (294) would then proceed by elimination of water, the mechanism requiring enolisation of the formyl group.

Equimolar quantities of aniline and α-cyano-β-ethoxy-N-formylacrylamide (291) when warmed together in alcoholic solution gave a yellow precipitate, m.p. 162°, the elemental analysis of which corresponded to the molecular formula of the acyclic derivative, α-cyano-β-anilino-N-formylacrylamide (293a) (C₉H₇N₃O₂). Mass spectrometry confirmed a mass ion of 215, corresponding to
the molecular formula C₇H₇N₃O₂ and the infra-red spectrum showed strong absorption at 2245 cm⁻¹ indicating the presence of a -CN group.

Cyclisation of the acyclic formylacrylamide(293a) to the deoxyuracil(294a) was attempted in several ways.

a) The first involved heating a sample of the acyclic compound above its melting point of 162°. The material recovered was found to be identical (i.r.) to the starting material.

b) The addition of sodium hydroxide to induce base catalysed cyclisation was found, in other cyclisations described later, to cause hydrolysis of the cyano group. Dilute ammonia solution was therefore used as the basic catalyst. The white powdery material, m.p. 220°, recovered from the reaction solution gave an elemental analysis much different to that required for the uracil (294a).

c) A sample of the acyclic derivative(293a) was heated with barium oxide, and a second sample was heated with calcium oxide, both attempts gave back the original starting material(293a) unchanged.

d) Finally a sample of the acyclic compound(293a) was treated with pyrophosphoryl chloride in carbon tetrachloride, in an attempt to forcibly dehydrate, and thus cyclise, the acyclic compound. Recovery of any crystalline material from the reaction mixture proved to be impossible. Cyclisation of the acyclic acrylamide(293a) was therefore not accomplished.

The formylacrylamide(291) solution, when treated with methylamine, gave a powdery white precipitate, which gave α-cyano-β-methylamino-N-formylacrylamide(293b) as plates, m.p.
Mass spectrometry indicated a mass ion of 153 which corresponded to the molecular formula $C_6H_7N_3O_2$ required for (293b) and the infra-red spectrum confirmed the presence of a $$-CN$$ group with strong absorption at 2240 cm$^{-1}$. Cyclisation techniques exactly similar to those described above for the anilino derivative(293a) were used, and similar results were obtained, the linear compound (293b) failed to cyclise to the uracil(294b).

With cyclohexylamine, a solution of the N-formyl reagent(291) produced a very dark solution which, even when evaporated to low volume, cooled and scratched, failed to crystallise. Rapid cooling to low temperature in the freezing box of a refrigerator gave a white precipitate which rapidly redissolved on warming. This precipitate, m.p. 170-180, was found to be rather sticky. Attempted recrystallisation failed to yield a crystalline material. The mass spectrum of the crude precipitate was found to contain a large peak at 194, and smaller peaks at 208 and 220. None of these corresponded to the required mass of $C_7H_8N_3O_2$ (293c) 221. A sample of the $\alpha$-cyano-$\beta$-cyclohexylamino-N-formylacrylamide(293c) was therefore not isolated.

2) Reactions of the N-acetyl reagent(292)

The reactions of $\alpha$-cyano-$\beta$-ethoxy-N-acetylacrylamide(292) with primary amines were intended to produce acyclic acetyl acrylamides of the type (295) which, by elimination of water, might be induced to cyclise to 2-deoxy-2-methyluracils(296), by an exactly similar reaction sequence to that proposed for the N-formylacrylamide reagent(291).

Treatment of an alcoholic solution of the acetylacrylamide
reagent(292) with aqueous ammonia gave a deep red coloured solution, which failed, on cooling, to yield a crystalline material. Evaporation to dryness gave a red oily solid, ethyl acetate extraction of which gave a green solution and a red crude solid. A small amount of crystalline material, m.p. 180-185, was recovered from the green solution which gave a mass ion of 315 (295a) required M, 153 and (296a) required M, 135. The red solid when recrystallised from water gave an almost colourless crystalline material, m.p. 243, the mass spectrum of which indicated the mass to be 135, which corresponded to the mass required for the uracil(296a) (C₆H₅N₃O). The infra-red spectrum of the crystals from the red solid indicated a strong absorption peak due to –CN at 2235 cm⁻¹ and elemental analysis confirmed the molecular formula to be C₆H₅N₃O. It would therefore appear that in this case the acyclic derivative, α-cyano-β-amino-N-acetyluracil(295a) spontaneously cyclised, during the reaction, to the deoxyuracil, 5-cyano-2-methyl-2-deoxyuracil(296a).

The anilinoacetylacrylamide(295b) was prepared by the reaction of aniline and the N-acetyl reagent(292) in alcoholic solution. Crystals of the acyclic anilino derivative(296a) were recovered from the reaction mixture and recrystallisation from methanol gave needles, m.p. 202-204 (decomp), of α-cyano-β-anilino-N-acetyl-acrylamide(295b). Elemental analysis confirmed the molecular formula to be C₁₇H₁₇N₃O₂. Cyclisation of (295b) was attempted as follows:

a) A suspension of the anilino derivative(295b) in hot water was treated with ammonium hydroxide solution. A colour change was noted in the suspended material and the product on recrystallisation
110

gave yellow needles, m.p. about 280° (decomp. before melting).
Elemental analysis of this material was much different to that
required for 5-cyano-1-phenyl-2-methyl-2-deoxyuracil(296b).
b) A second sample of (295b) was maintained above its melting
point at 230° for 4-5 min. Severe decomposition occurred and the
resulting tar proved impossible to purify.

Cyclisation of the acyclic anilinoacetylacrylamide(295b) was
therefore, as with the N-formyl derivatives (293a) and (293b)
and (293c), found not to be possible.

3) The thiazine reagent (72)

5-Cyano-2-ethylthio-4-oxo-1,3-thiazine(72), when reacted
with primary amines gave the linear derivative, ethyl N-(α-
cyano-β-alkyl(aryl)aminoacryloyl)dithiocarbamate(297). Cyclisation
of this by elimination of ethanthiol gave the 5-cyano-1-alkyl-
(aryl)-2-thiouracil(298).

The thiazine reagent(72), when treated with aniline gave
crystals of ethyl N-(α-cyano-β-anilinoacryloyl)dithiocarbamate(297a)
which gave yellow laths, m.p. 154° (resolidified and then 270°),
from ethanol. Elemental analysis of (297a) corresponded to the
molecular formula required for the acyclic dithiocarbamate and
its mass spectrum showed a mass ion of 291, which was again in
agreement with the required formula, C13H13N3O5S, of (297a). The
i.r. spectrum of (297a) showed intense -CN absorption at 2200 cm⁻¹.

Cyclisation of (297a) was initially attempted by dissolution of
the solid in 0.5 M sodium hydroxide solution maintained at 80-
100° for 10 min, followed by neutralisation with mineral acid.
A white precipitate was produced, m.p. 284° (decomp), however, the
infra-red spectrum of this compound was found to show no band characteristic of -CN. That hydrolysis of the 5-cyano group to a 5-carboxamido group had occurred in the alkaline solution to give 5-carboxamido-1-phenyl-2-thiouracil (299) was confirmed by elemental analysis. The u.v. spectrum of (299) showed an upfield alkali shift, from $\lambda_{\text{max}}$ 311 nm in neutral solution to $\lambda_{\text{max}}$ 323 nm at pH 10. Similarly, treatment of the anilinodithiocarbamate (297a) with cold 0.5 M sodium hydroxide solution, for 5 min, or with cold 0.25 M sodium hydroxide solution for 5 min, also caused hydrolysis of the cyano group, resulting in the formation of the 5-carboxamidothiouracil (299).

When a sample of (297a) was dissolved in 0.25 M sodium hydroxide solution followed by immediate neutralisation of the solution with mineral acid, the product precipitated (298a), m.p. 280°, and was found to give strong absorption in the i.r. at $\nu_{\text{max}}$ 2205 cm$^{-1}$ (CN). Elemental analysis of (298a) confirmed it to be 5-cyano-1-phenyl-2-thiouracil. The u.v. spectrum of (298a) showed a downfield alkali shift from $\lambda_{\text{max}}$ 312 nm in neutral solution to $\lambda_{\text{max}}$ 294 nm at pH 10.

Both the 5-carboxamidothiouracil (299) and the 5-cyanothiouracil (298a) were subjected to reduction with Raney nickel in attempts to desulphurise the rings, and so produce 5-carboxamido-1-phenyl-2-deoxyuracil (300) and 5-cyano-1-phenyl-2-deoxyuracil (294a) respectively. An alcoholic solution of the amide derivative (299) when treated with Raney nickel gave a white precipitate, from which 5-carboxamido-1-phenyl-2-deoxyuracil (300) was obtained as pale green laths, m.p. 264°, the formula of which was confirmed by elemental analysis. The u.v. spectrum of (300) was found to differ considerably from that of the 2-thiouracil (299). The alkali shift
due to the presence of the enolisable 2-thio and 4-oxo ring substituents in the original thiouracil compound (299) was not observed in the spectrum of the desulphurised material. The u.v. spectrum of (300) showed an absorption peak at $\lambda_{\text{max}}$ (pH 7 and pH 10), 314 nm. (With the removal of the 2-thio substituent in the compound (300), no enol tautomer is possible in the 4-position).

Raney nickel desulphurisation of 5-cyano-1-phenyl-2-thiouracil (298a) was followed by u.v. studies. During a period of 1.5 h, the u.v. spectrum of the 2-thiouracil (298a), which showed absorption maxima at $\lambda_{\text{max}}$ (pH 7), 312 nm and $\lambda_{\text{max}}$ (pH 10), 294 nm, thereby exhibiting a strong downfield alkali shift, changed to a spectrum with absorption maxima at $\lambda_{\text{max}}$ (pH 7), 320 nm and $\lambda_{\text{max}}$ (pH 10), 323 nm. The solution, on cooling, gave crystals, m.p. 280. The mass spectrum of these crystals indicated a mass ion of 197, which corresponded to the mass required for 5-cyano-1-phenyl-2-deoxyuracil (294a). Elemental analysis confirmed the molecular formula to be $\text{C}_{11}\text{H}_{7}\text{N}_{3}\text{O}$. The i.r. spectrum of (294a) showed strong CN absorption at $\nu_{\text{max}}$ 2210 cm$^{-1}$.

The reaction between the thiazine reagent (72) and cyclohexylamine in benzene gave an oil, which proved most reluctant to crystallise. When the experiment was repeated using methanol as solvent, chunky crystals, m.p. 174 (then resolidified and remelted at 264). The i.r. spectrum of this crystalline material showed intense absorption at $\nu_{\text{max}}$ 2210 cm$^{-1}$(CN), and the mass spectrum indicated a mass ion of 297 corresponding to the formula of $\text{C}_{15}\text{H}_{9}\text{N}_{3}\text{OS}_{2}$ required for ethyl N-(α-cyano-β-cyclohexylaminoacryloyl)dithiocarbamate (297b).

Cyclisation and hydrolysis of (297b) in warm 0.5 M sodium
\[(291) \xrightarrow{\text{R.NH}_2} (293) \xrightarrow{\text{R.NH}_2} (294)\]

\[a) \ R = \text{Ph} \]
\[b) \ R = \text{Me} \]
\[c) \ R = \text{C}_6\text{H}_{12} \]

\[(292) \xrightarrow{\text{R.NH}_2} (295) \xrightarrow{\text{R.NH}_2} (296)\]

\[a) \ R = \text{H} \]
\[b) \ R = \text{Ph} \]

\[(300) \xrightarrow{\text{R.NH}_2} (297) \xrightarrow{\text{R.NH}_2} (298) \xrightarrow{\text{R.NH}_2} (299) \xrightarrow{\text{R.NH}_2} (300) \]

\[(299) \ R = \text{Ph} \]
\[(300) \ R = \text{C}_6\text{H}_{12} \]
hydroxide solution followed by neutralisation with mineral acid, gave a precipitate, which gave plates, m.p. 270°, from ethyl acetate. No -CN absorption was found in the i.r. spectrum and the mass ion was found to be 253 which corresponded to the mass of 5-carboxamido-1-cyclohexyl-2-thiouracil (301). The u.v. spectrum of (301) showed absorption peaks at λmax 323 nm, in neutral solution, and λmax 305 nm at pH 10 with the downfield alkali shift being due to the presence of the enolisable 2-thio and 4-oxo groups on the ring.

4) Reactions of the N-methylacryloylcarbamate reagent (67)

3-Methyl substituted 5-cyanouracils were prepared by the reaction between ethyl N-(α-cyano-β-ethoxy-N-methylacryloyl)-carbamate (67) and various primary amines, giving initially the ethyl N-(α-cyano-β-alkyl(aryl)amino-N-methylacryloyl)carbamate (302), which by elimination of ethanol cyclised to the 5-cyano-3-methyl-1-alkyl(aryl)uracil (303).

The amines used in this section were, p-chlorobenzylamine (304); o-chlorobenzylamine (305); imidazole-4-ethylamine hydrochloride (histamine:hydrochloride) (306); p-hydroxyphenylethylamine hydrochloride (Tyramine hydrochloride) (307); 3,4-dihydroxyphenylethylamine hydrochloride (3-hydroxytyramine) (308) and indole-3-ethylamine hydrochloride (Tryptamine:hydrochloride) (309).

An alcoholic solution of p-chlorobenzylamine (304) and ethyl N-(α-cyano-β-ethoxy-N-methylacryloyl)carbamate (67) after heating under reflux for 15 min, readily gave a fine white crystalline material (311), m.p. 181°. Spectroscopic studies of (311) showed strong CN absorption at 2235 cm⁻¹, u.v. absorption typical of the 3-methyluracil system at λmax (pH 7) 279 and 224 nm.
$\lambda_{\text{max}}(\text{pH}10)$, 276 and 222 nm and a mass ion of 275 which corresponded to 5-cyano-3-methyl-1-p-chlorobenzyluracil (311) ($C_{13}H_{11}N_3O_3Cl$). Elemental analysis confirmed this formula. The acyclic intermediate ethyl N-(α-cyano-β-chlorobenzylamino-N-methylacryloyl)carbamate (310) was not recovered, cyclisation presumably occurred during the reaction sequence.

By an exactly similar reaction sequence, the o-chloro derivative 5-cyano-3-methyl-1-o-chlorobenzyluracil (312) was prepared from the N-methylcarbamate (67) and o-chlorobenzylamine (305), except that during the heating process, as cyclisation of the carbamate intermediate occured, a precipitate of (312) was produced. This was collected and gave (312) as plates, m.p. 160$^\circ$, from nitromethane. Spectroscopic studies showed -CN absorption at 2230 cm$^{-1}$ in the i.r., u.v. absorption at $\lambda_{\text{max}}$(pH7), 278 and 224 nm and $\lambda_{\text{max}}$(pH10), 274 and 224 nm and a mass ion at 275 corresponding to the required formula for (312), with a large secondary peak at 240, indicating loss of -Cl.

The remaining amines were available only in the form of their hydrochlorides and were released by treatment with sodium ethoxide, prior to use.

The reaction between histamine (4-imidazoleethylamine) (306) and ethyl N-(α-cyano-β-ethoxy-N-methylacryloyl)carbamate (67) gave on dilution with ether, an oil, which crystallised on standing. This material (313) gave needles, m.p. 196$^\circ$, from nitromethane. The i.r. spectrum of (313) showed -CN absorption at 2240 cm$^{-1}$; the u.v. spectrum showed absorption peaks at $\lambda_{\text{max}}$(pH7), 285 and 228 nm and at $\lambda_{\text{max}}$(pH10), 283 and 228 nm and mass spectrometry indicated a very strong mass ion at 245, which corresponded to
that required for 5-cyano-3-methyl-1-(4'-imidazoleethyl)uracil (313). The acyclic intermediate was not isolated.

The solution containing tyramine (p-hydroxyphenylethylamine) (307), and the N-methylacryloylcarbamate(67) gave crystals, m.p. 198, of the cyclised product, 5-cyano-3-methyl-1-(4'-hydroxyphenylethyl)uracil(314) which showed strong -CN absorption at 2240 cm⁻¹.

The mass spectrum of (314) showed a mass ion of 271, corresponding to that required for (314). The u.v. spectrum showed absorption peaks at λmax 285 and 217 nm in neutral solution and at λmax 286 and 216 nm at pH 10. The structure of 5-cyano-3-methyl-1-(4'-hydroxyphenylethyl)uracil(314) was confirmed by elemental analysis.

The deeply coloured solution of 3-hydroxytyramine (3,4-dihydroxyphenylethylamine)(308) when treated with the N-methyl reagent (67), rapidly gave a heavy precipitate, on warming, from which 5-cyano-3-methyl-1-(3', 4'-dihydroxyphenylethyl)uracil(315) was obtained as needles, m.p. 204. Spectroscopic studies of (315) showed strong -CN absorption at 2243 cm⁻¹, u.v. absorption at λmax (pH 7) 288 and 231 nm and at λmax (pH 10), 284 and 228 nm, and a mass ion of 287, corresponding to that required for (315).

The solution containing tryptamine (indole-3-ethylamine)(309) and the N-methyl reagent(67) gave an immediate precipitate. The mixture was gently warmed to ensure complete cyclisation, and the thick white deposit gave 5-cyano-3-methyl-1-(indole-3'-ethyl) uracil(316) as plates, m.p. 236, from water. The i.r. spectrum of (316) showed -CN absorption at 2238 cm⁻¹, its u.v. spectrum indicated absorption peaks at λmax (pH 7), 279 and 223 nm and at λmax (pH 10), 276 and 215 nm and its mass ion was found to correspond to that required by the structure assigned to (316), of 284.
It is of interest to note that on no occasion was an acyclic intermediate of the type (302) recovered, even though the most vigorous reaction conditions used involved merely heating under reflux in ethanol. It is possible, however, that small traces of sodium ethoxide might have been present in the alcoholic solutions of the amines liberated from their hydrochloric salts. This would account for the rapid precipitation of the uracil derivatives (314), (315) and (316) from their reaction solutions.

The u.v. spectra of the uracils prepared from the thiazine(72) and N-methylacryloylcarbamate(67) reagents are displayed in figs. 20 and 21 respectively. It may be seen that those uracils unsubstituted at N₃, formed from the thiazine reagent (fig. 20) characteristically absorbed in the u.v. at around \( \lambda_{\text{max}} \) 323-311 nm in neutral solution, whereas the 3-methyluracils (fig. 21) characteristically absorbed at around \( \lambda_{\text{max}} \) 288-278 nm with a second absorption peak in the region \( \lambda_{\text{max}} \) 231-217 nm.

It may also be seen that the pronounced alkaline shifts observed with the 2-thiouracils (298a) and (299) are duplicated, but to a much lesser degree by the 3-methyluracils (311)-(316). It would therefore appear that the 2-thiouracil ring system is much more readily enolised, in the presence of alkali, to the 2-mercapto-4-hydroxy tautomer, than the uracil ring system is enolised to its 2,4-dihydroxy tautomer.

No alkaline shift was observed in the desulphurised 2-deoxyuracils (300) and (294a) as the 4-oxo group in these molecules is no longer enolisable to the tautomeric 4-hydroxy isomer.
Fig. 20 I.r. and u.v. data for the uracil derivatives of the thiazine reagent (72) ($\nu_{\text{max}}$ values quoted in cm and $\lambda_{\text{max}}$ values quoted in nm).

<table>
<thead>
<tr>
<th>Compound reference</th>
<th>I.r. absorption</th>
<th>U.v. absorption pH 7</th>
<th>U.v. absorption pH 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>297a</td>
<td>2200</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>298a</td>
<td>2205</td>
<td>312</td>
<td>294</td>
</tr>
<tr>
<td>299</td>
<td>-</td>
<td>311</td>
<td>323</td>
</tr>
<tr>
<td>300</td>
<td>-</td>
<td>314</td>
<td>314</td>
</tr>
<tr>
<td>293a</td>
<td>2210</td>
<td>320</td>
<td>323</td>
</tr>
<tr>
<td>297b</td>
<td>2210</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>301</td>
<td>-</td>
<td>323</td>
<td>305</td>
</tr>
</tbody>
</table>

Fig. 21 I.r. and u.v. data for the uracil derivatives of the N-methyl reagent (67) ($\nu_{\text{max}}$ values quoted in cm$^{-1}$, and $\lambda_{\text{max}}$ values quoted in nm).

<table>
<thead>
<tr>
<th>Compound reference</th>
<th>I.r. absorption</th>
<th>U.v. absorption pH 7</th>
<th>U.v. absorption pH 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>311</td>
<td>2235</td>
<td>279</td>
<td>276</td>
</tr>
<tr>
<td>312</td>
<td>2230</td>
<td>278</td>
<td>274</td>
</tr>
<tr>
<td>313</td>
<td>2240</td>
<td>285</td>
<td>283</td>
</tr>
<tr>
<td>314</td>
<td>2240</td>
<td>285</td>
<td>286</td>
</tr>
<tr>
<td>315</td>
<td>2243</td>
<td>288</td>
<td>284</td>
</tr>
<tr>
<td>316</td>
<td>2238</td>
<td>279</td>
<td>276</td>
</tr>
</tbody>
</table>
CHAPTER NINE

The synthesis of pyrazolo[3,4-d]pyrimidine-4-ones and pyrazolo[3,4-d]pyrimidine-4,6-diones

Pyrazolo[3,4-d]pyrimidines are purine analogues, and as such have useful properties as antimetabolites in purine biochemical reactions. Of special interest is the hypoxanthine analogue (93a), (allopurinol), which is a known inhibitor of xanthine oxidase in vivo; this property makes the compound valuable in the treatment of gout and related diseases, in which diminished oxidation of purines is required.

Other related pyrazolo[3,4-d]pyrimidines, including the xanthine analogue (94a) (oxyallopurinol) and the thiohypoxanthine analogue (317) (thiopurinol), have a similar inhibitory effect on xanthine oxidase. Many compounds of this type also have a marked antitumour and antileukemic activity.

A facile synthesis of substituted uracils was proposed by Shaw, in which ethyl N-(α-cyano-β-ethoxyacryloyl)carbamate (124) gave, on reaction with a primary amine, an acyclic carbamate of the form (318) which could be induced to cyclise, with a subsequent loss of ethanol, to give a 5-cyanouracil of the type (319).

The reaction of the ethoxycarbamate (124) with phenylhydrazine
however, was found to be anomalous, in that, although the crystalline compound first formed was isomeric with the acyclic aminomethylene derivative (125), unlike derivatives of this type, the compound had no CN absorption in its i.r. spectrum. Mild alkaline hydrolysis of the substance readily gave a second substance, elemental analysis of which suggested that a molecule of ethanol had been lost. In view of these results it was decided to reinvestigate the reaction. It had been suggested earlier that an acyclic aminomethylene derivative was first formed and that this cyclised to the anilinouracil (126) when heated or warmed with alkali, but at the time no spectroscopic evidence supporting these conclusions had been presented.

The first reaction product of (124) and phenylhydrazine was indeed the acyclic aminomethylene derivative (125). Its structure was confirmed by elemental analysis and i.r. spectra, which showed the presence of the CN group. However, when the compound was recrystallised from ethanol it isomerised to a compound (130) which had no band characteristic of CN in its i.r. spectrum. When either this or the acyclic compound (125) was heated at 160 °C for a few minutes, ethanol was lost, and another crystalline compound was isolated with no CN band in the i.r. spectrum and identical with the material previously regarded as the anilinouracil (126). It is suggested that the sequence of reactions is best explained as follows.

The acyclic derivative (125) isomerises to the aminopyrazole (130), which when heated or treated with alkali is cyclised to the pyrazolo[3,4-d]pyrimidine (94c).

We have been particularly interested to see if reactions of
this type might be extended to include more general syntheses of pyrazolo[3,4-d]pyrimidines of use, for example, in the preparation of unsubstituted compounds such as allopurinol(93a) and oxyallopurinol(94a).

Comparison samples of the pyrazolo[3,4-d]pyrimidines prepared in this section were made using methods developed by Robins et al and discussed in some detail in the introductory section.

Hydrazine reacted rapidly with the ethoxycarbamate derivative (124) to produce an immediate precipitate of the hydrazinomethylene derivative(320), which, when briefly heated at 140°, cyclised directly to pyrazolo[3,4-d]pyrimidine-4,6-dione, oxyallopurinol (94a), presumably via the intermediate pyrazole(321), although this was not isolated from this reaction.

In contrast methylhydrazine and the ethoxycarbamate(124) produced directly, crystals of 5-amino-1-methylpyrazole-4-(N-ethoxy-carbonyl)carboxamide(323), which unlike (320) showed no CN absorption in its i.r. spectrum, and moreover could be diazotised and coupled by use of the Bratton Marshall reagents. The intermediate acyclic compound (322) was indeed formed in the original reaction mixture. The i.r. spectrum of this solution indicated the presence of strong CN absorption which slowly faded prior to crystallisation of the aminopyrazole(323). When the aminopyrazole(323) was heated at 160° for a short time, it rapidly cyclised with evolution of ethanol to give 1-methylpyrazolo[3,4-d]pyrimidine-4,6-dione(94b) as an amorphous powder, which gave crystals from water, m.p. 310°.

Comparison was made with the original phenylhydrazine reaction with the ethoxycarbamate derivative(124) which, in alcoholic solution gave a heavy white precipitate of ethyl N-(α-cyano-β-
phenylhydrazineacryloyl)carbamate(125), which upon heating, melted at about 200°, effervesced, and resolidified (to melt again at 310° (decomp)). 1-Phenylpyrazolo[3,4-d]pyrimidine-4,6-dione(94c) gave plates from this resolidified material, m.p. 320°. The original precipitate(125) was assigned the structure shown, as a result of spectroscopic studies where strong CN absorption in the i.r. was found at \( \gamma_{\text{max}} \) 2239 cm\(^{-1}\), and also in view of the absence of strong colouration when the material was treated with nitrous acid and the Bratton Marshall reagents.

Recrystallisation of (125) from ethyl acetate gave needles (130), which with diazotization and coupling with the Bratton Marshall reagents gave a highly coloured material, with u.v. absorption at \( \lambda_{\text{max}} \) 494 nm. The methyl derivative (323) with the same reagents gave an absorption peak at \( \lambda_{\text{max}} \) 483 nm and recrystallisation of the acyclic hydrazine derivative(320) from ethyl acetate gave a crystalline material(321), which, with the Bratton Marshall reagents, gave an absorption peak at 490 nm. It is to be assumed, therefore, that recrystallisation of the acyclic hydrazino derivative(320) and the acyclic phenylhydrazino derivative(125) gave, by intramolecular rearrangement, the substituted pyrazoles (321) and (130), analogous to the methyl derivative(323) produced in situ, which were responsible for the u.v. absorptions obtained with the Bratton Marshall reagents.

The progress of the second cyclisation stage from the pyrazole to the pyrazolo[3,4-d]pyrimidine ring system, involving loss of ethanol, was followed by u.v. studies, as strong absorption peaks in the range \( \lambda_{\text{max}} \) 238-247 nm were produced, characteristic of this type of ring system (fig. 22).
Fig. 22 U.v. data for the hydrazine and substituted hydrazine derivatives of ethyl N-(α-cyano-β-ethoxycarbonylacryloyl)carbamate (124) (\( \lambda_{\text{max}} \) values quoted in nm).

<table>
<thead>
<tr>
<th>Hydrazine used</th>
<th>Acyclic hydrazino acrylamide</th>
<th>Pyrazole intermediate pH 10</th>
<th>Bratton Marshall test</th>
<th>Pyrazolo-pyrimidine pH 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrazine hydrate</td>
<td>282 216</td>
<td>261</td>
<td>490</td>
<td>268 243</td>
</tr>
<tr>
<td>Methyl hydrazine</td>
<td>278 212</td>
<td>263</td>
<td>483</td>
<td>269 247</td>
</tr>
<tr>
<td>Phenyl hydrazine</td>
<td>-</td>
<td>264</td>
<td>494</td>
<td>- 248</td>
</tr>
</tbody>
</table>
The structures of the above pyrazolopyrimidines were confirmed by elemental analysis, mass spectrometric studies and comparison with samples prepared by the route developed by Cheng and Robins, in which the appropriate monosubstituted hydrazine was reacted with ethoxymethylenemalononitrile, to yield the corresponding 1-substituted-5-amino-4-cyanopyrazole (91), which when treated with cold concentrated sulphuric acid gave the corresponding 1-substituted-5-aminopyrazole-4-carboxamide (92).

Fusion of the carboxamide (92) with urea gave the corresponding 1-substituted pyrazolo[3,4-d]pyrimidine-4,6-dione (94) and, as required later in this section, fusion of the carboxamide (92) with formamide gave the corresponding 1-substituted pyrazolo-[3,4-d]pyrimidine-4-ones (93).

The structure of 5-amino-1-phenylpyrazole-4-carboxamide (92c) was established by Cheng and Robins, by an unambiguous synthesis of (92c) from 5-hydroxy-1-phenylpyrazole-4-ethylcarboxylate (89) by treatment with phosphoryl chloride and phosphorous pentachloride to give 5-chloro-1-phenylpyrazole-4-ethylcarboxylate (324), which with alcoholic ammonia in a bomb at 170° gave 5-amino-1-phenylpyrazole-4-carboxamide (92c).

Diazotisation and coupling of the 5-aminopyrazole (92a) and 5-amino-1-phenylpyrazole-4-carboxamide (92c) with the above Bratton Marshall reagents gave derivatives with u.v. absorption peaks of 512 nm and 522 nm respectively.

An alternative route to the 5-aminopyrazole-4-carboxamides was also investigated. This involved the reaction of cyanoacetamide with formamidine hydrochloride to give aminoethylenecyanoacetamide (325) which, with hydrazine or a mono-substituted hydrazine
gave the appropriate pyrazole-4-carboxamide (92). The product of this reaction was found to be identical with that produced by hydrolysis of the 4-cyanopyrazole (91).

The 1-substituted pyrazolo[3,4-d]pyrimidine-4,6-diones (94a), (94b) and (94c) were found to be identical (m.p., mixed m.p., t.l.c. u.v.) with the pyrazolo[3,4-d]pyrimidines prepared by the alternative route described above from urea and the 5-aminopyrazole-4-carboxamides (92a), (92b) and (92c) respectively.

Pyrazolo[3,4-d]pyrimidine-4-ones

The intermediate ethoxyacrylamide (291) required for similar syntheses of pyrazolopyrimidines of the allopurinol (93a) type, was prepared as follows. Treatment of cyanoacetic acid with formamide in the presence of acetic anhydride gave the crystalline cyanoacetylformamide (326), and further treatment of this with triethyl orthoformate and acetic anhydride gave, on evaporation, a syrup, which rapidly crystallised to give the ethoxymethylene derivative, $\alpha$-cyano-$\beta$-ethoxy-N-formylacrylamide (291). The last compound could be obtained directly (and to advantage), by combining the two reactions and omitting isolation of the intermediate (326).

Recrystallisation of the N-formylacrylamide (291) from methanol resulted in hydrolysis of the formyl group to give $\alpha$-cyano-$\beta$-ethoxy-acrylamide (327). Hydrolysis did not occur when the less convenient solvent system, ethyl acetate-petroleum ether was used.

Treatment of the N-formylacrylamide (291) with hydrazine in ethanol produced an immediate pale yellow precipitate, in almost quantitative yield, of the hydrazinomethyleneacrylamide (328), which still retained the CN group (strong absorption in the i.r.
at 2240 cm\(^{-1}\) and which failed to yield a strongly coloured product with the Bratton Marshall reagents.

When the hydrazinomethylene acrylamide(328) was briefly heated at 150\(^{\circ}\) there was little or no visible change in the material but the residue was found to be the pyrazolopyrimidine, allopurinol(93a), formed in high yield, and readily recrystallised by dissolution in sodium hydroxide solution followed by neutralisation with hydrochloric acid. It was identified by comparison with an authentic specimen prepared by the route devised by Cheng and Robins, in which a mixture of formamide and 5-amino-pyrazole-4-carboxamide(92a) was fused together by boiling the mixture, to give pyrazolo[3,4-d]pyrimidine-4-one(93a).

Under the conditions used for cyclisation of the hydrazinomethylene derivative(328), it was not possible to isolate the intermediate aminopyrazole(329), which was presumably formed by rearrangement, prior to cyclisation. Recrystallisation of the acyclic derivative(328) from nitromethane gave a substance with no CN absorption but which with the Bratton Marshall reagents, gave a highly coloured derivative with u.v. absorption at \(\lambda_{\text{max}}\) 520 nm, assumed to be 5-aminopyrazole-4-(N-formyl)carboxamide (329). This material when heated to 170\(^{\circ}\) for 10 min gave a residue identical (m.p., mixed m.p., u.v.) with the pyrazolopyrimidine(93a) prepared by heating the hydrazinomethylene acyclic derivative(328).

In a similar manner, treatment of the formylacrylamine(291) with phenylhydrazine gave the acyclic compound \(\alpha\)-cyano-N-formyl-\(\beta\)-phenylhydrizinoacrylamide(330) which absorbed strongly in the i.r. at \(\nu_{\text{max}}\) 2240 cm\(^{-1}\)(CN) and which failed to give a strongly coloured derivative with the Bratton Marshall reagents. When recrystallised from nitromethane the acyclic compound(330)
readily cyclised to the aminopyrazole (331) which showed no CN absorption in the i.r., and which gave a strongly coloured ($\lambda_{\text{max}}$ 531 nm) derivative with the Bratton Marshall reagents.

When heated to 220° for 10 min, 5-amino-1-phenylpyrazole-4-(N-formyl)carboxamide (331) melted, effervesced and resolidified. The residue of 1-phenyl-pyrazolo[3,4-d]pyrimidine-4-one (1-phenyl-allopurinol) (93c) formed needles, m.p. 298–299° from water, and was identified by elemental analysis and by comparison with an authentic sample prepared from compound (92c) and formamide. Desulphurisation of the related 6-thiopyrazolopyrimidine (332) (prepared later) with Raney nickel also gave compound (92c).
The synthesis of 4-hydroxy-6-mercaptopyrazolo[3,4-d]pyrimidines

Thio-compounds analogous to (332) were prepared by the reaction between hydrazine or a substituted hydrazine and the cyanothiazine (72). This in turn was prepared by reaction between cyanoacetic acid and ethyl dithiocarbamate in acetic anhydride, which at 25°C gave a readily separable mixture of ethyl N-cyanoacetyldithiocarbamate (333) and ethyl N-acetyldithiocarbamate (334). Reaction of the cyanoacetyl derivative (333) with triethyl orthoformate in acetic anhydride gave 5-cyano-2-ethylthio-4-oxo-1,3-thiazine (72), presumably by cyclisation of the acyclic cyanoacetyldithiocarbamate (335).

The thiopyrazolopyrimidine (332) was prepared by the reaction of phenylhydrazine with the cyanothiazine (72) in ethanol to give initially the acyclic dithiocarbamate (336) which, when heated at 140°C for 10 min under a nitrogen atmosphere, melted, with the evolution of ethanthiol, and then resolidified. The mercaptopyrazolopyrimidine, 4-hydroxy-6-mercaptopyrazolo[3,4-d]pyrimidine (332), gave needles from ethanol with u.v. absorption at λmax 313 and 254 nm in neutral solution and λmax 318 and 253 nm at pH 10.

It was found that if a sample of (336) was heated at 180°C for 30 min in air, then an amorphous yellow solid was obtained, with u.v. absorption at λmax 285 and 262 nm (no change in alkali). This absorption was found to be similar to the values obtained for 6-chloro-4-hydroxy-1-phenylpyrazolo[3,4-d]pyrimidine (97c)
prepared elsewhere, suggesting that the compound (338), showing no alkali shift in its u.v. spectrum, might be the disulphide of the mercaptopyrazolopyrimidine (332). A sample identical (mixed m.p. and u.v.) with the suggested disulphide (338) was obtained when a small amount of the acyclic derivative (336) was warmed gently in 0.5M aqueous sodium hydroxide solution, the reaction being followed by u.v. absorption spectra. After 2.5 h the u.v. spectra of the two samples were identical.

Recrystallisation of the acyclic compound (336) from nitromethane gave the aminopyrazole, which when heated at 140° also gave the mercaptopyrazolopyrimidine (332). The aminopyrazole (337) showed no CN absorption in its i.r. spectrum, gave a positive Bratton Marshall test (λmax 514 nm) and had u.v. absorption maxima at 312 and 267 nm. Whereas the acyclic dithiocarbamate (336) gave strong CN absorption at λmax 2210 cm⁻¹, no coloured derivative in the Bratton Marshall test and had diffuse u.v. absorption from 225-275 nm. Reaction of the thiazine (72) with phenylhydrazine at temperatures in excess of 5° gave the aminopyrazole (339) directly.

The structure of the mercaptopyrazolopyrimidine (332) was confirmed by elemental analysis, mass spectrometry and by its conversion, with hot aqueous chloroacetic acid, into the dioxo-derivative (94c) which had previously been prepared by fusion of the aminopyrazole (92c) and urea or (as in our own studies mentioned earlier), by a sequence of reactions from the ethoxy-carbamate (124) and phenylhydrazine. The structure of (332) was further confirmed by its conversion, by Raney nickel reduction, into 1-phenylallopurinol (93c) which had previously been prepared by fusion of the aminopyrazole (92c), with formamide or (as in...
our own studies mentioned earlier) by a sequence of reactions from the N-formylacrylamide(291) and phenylhydrazine.

Thio compounds analogous to (332) have previously been prepared by heating a chloropyrazolo-pyrimidine(96) or (97) with thiourea, or potassium hydrosulphide, or by fusion of the 5-amino-pyrazole-4-carboxamide(92) with thiourea. Both of these routes were tried in an effort to produce a second specimen of the pyrazolopyrimidine(332) for direct comparison.

The dioxo-derivative(94c) was treated with phosphoryl choride and phosphorous trichloride to yield the dichloro derivative(96c) which in turn gave, with aqueous potassium hydroxide solution, the 6-chloro-4-hydroxy derivative(97c). This derivative was heated to reflux in ethanolic solution with thiourea and again heated as an intimate mixture with thiourea, for 2 h at 210°. In both cases the monochloro derivative was recovered unchanged.

An intimate mixture of the pyrazole(92c) and thiourea was heated at 210° for 2 h. Extensive degradation occurred, however, u.v. studies failed to detect any mercaptopyrazolopyrimidine formation.

The 1-methyl analogue of the mercaptopyrazolopyrimidine(332) namely 4-hydroxy-6-mercapto-1-methylpyrazolo[3,4-d]pyrimidine (341) was prepared in a similar manner to the 1-phenyl derivative above. Solutions of the thiazine(72) and methylhydrazine, both in nitromethane, were mixed and gave a pale yellow precipitate on standing overnight, which gave a weak absorption peak in the i.r. spectrum at $\nu_{\text{max}}$ 2230 cm$^{-1}$, as well as a positive Bratton Marshall test ($\lambda_{\text{max}}$ 504 nm).
Rearrangement of the acyclic derivative (339) portion of this mixture to the methylpyrazole (341) occurred as the solid was recrystallized. 5-Amino-1-methylpyrazole-4-[(N-ethylthiothiocarbonyl)-carboxamide (341) when heated at 240 °C under a nitrogen atmosphere, melted, effervesced (the gas evolved gave a precipitate with alkaline potassium permanganate solution, indicating it to be an oxidizable gas) and resolidified. A small amount of sublimate was obtained (λ max 237 and 270 nm) which was found to be different to the solid residue (λ max 247 and 275 nm).

The solid residue was purified by dissolution in aqueous potassium hydroxide solution followed by reprecipitation with glacial acetic acid. 4-Hydroxy-6-mercapto-1-methylpyrazolo[3,4-d]pyrimidine (341) was obtained as a white solid, m.p. > 300 °C, which was found to be identical to a sample prepared by the route suggested by Cheng and Robins, by fusion of an aminopyrazole carboxamide and thiourea. An intimate mixture of 5-amino-1-methylpyrazole-4-carboxamide (92b) and thiourea was heated at 210 °C for 2 h. The fused product was purified as above.

A second route involving the fusion of a mixture of 6-chloro-4-hydroxy-1-methylpyrazolo[3,4-d]pyrimidine (97b) with thiourea also gave a product identical to the mercapto derivative (341). The 6-chloro derivative (97b) was prepared by a similar route to that used for the 1-phenyl derivative (97c), in which the dioxo-1-methylpyrazolo[3,4-d]pyrimidine (94b) was treated with phosphoryl chloride and phosphorous trichloride to give the dichloro compound (96b) which, in turn gave (97b) by alkaline hydrolysis with potassium hydroxide.
U.v. analysis of the products of both confirmatory routes showed absorption at \( \lambda_{\text{max}} \) 247 and 275 nm. This is in agreement with the spectrum for (341) prepared from the thiazine and methyl hydrazine, but differs from the quoted values of Cheng and Robins, of absorption \( \lambda_{\text{max}} \) 239 and 278 nm. In view of this discrepancy the structure of (341) was further confirmed by its reduction with Raney nickel in ethanol, to 1-methylallopurinol (93b) (\( \lambda_{\text{max}} \) 252 nm). The product of this reaction being identical (u.v., m.p., mixed m.p.) to an authentic sample prepared by fusion of formamide and 5-amino-1-methylpyrazolo-4-carboxamide(92b).

The reaction of the thiazine(72) with hydrazine was examined in an attempt to prepare 4-hydroxy-6-mercaptopyrazolo[3,4-d]pyrimidine(344) but was found to be rather difficult. A product precipitated with absorption at 2230 cm\(^{-1}\) (CN) in the i.r. spectrum, which was also present after the sample had been heated at 190° for 15 min. Thin layer chromatography indicated that the fused sample consisted of a number of compounds, one of which corresponded to an authentic sample of the thiopyrazolopyrimidine (344), prepared by a fusion reaction between 5-aminopyrazolo-4-carboxamide(92a) and thiourea. This correlation was confirmed by i.r. analysis of the appropriate compound separated by column chromatography.

Purification of the bulk of the fused residue was attempted using a number of solvent systems, namely, nitromethane, ethanol, dioxan, and aqueous sodium hydroxide and acetic acid. None of these however, gave an analytically pure sample of the required product.

The original precipitate was found to give a positive Bratton Marshall test (\( \lambda_{\text{max}} \) 508 nm) which would appear to indicate that
some cyclisation to the pyrazole ring structure had occurred. This together with the \(-CN\) absorption in the i.r. suggests some sort of mixture, but not one containing the acyclic derivative(342) as the sole cyano- containing molecule, for (342) should rearrange on heating to give the pyrazole(343) with the subsequent loss of \(-CN\) absorption in the i.r.

One suggestion is that the unsubstituted hydrazine upon reaction with the thiazine(72), forms the acyclic carbamate(342), which, itself possessing a primary amine group, further reacts with a second thiazine molecule to form the intermediate(345), which on cyclisation, would give the pyrazole(346), which would give both a positive Bratton Marshall test and also a permanent \(-CN\) absorption in the i.r.

This suggestion is of course speculative and more work would be required to be carried for it to be acceptable. The presence of the primary amine group in (342) however, must contribute largely to the multiplicity of products from this reaction.

A similar suggestion could perhaps account for the relatively low yields obtained, when the phenyl, and methyl derivatives (332) and (341) respectively, are prepared by reaction of the thiazine (72) with the corresponding substituted hydrazines, at room temperature and above room temperature. Under these conditions the acyclic carbamates (336) and (339) would rapidly cyclise to the 5-amino pyrazoles(337) and (340) respectively, which in turn could react with a second molecule of the thiazine(72) to give the pyrazoles(347) and (348) respectively.
CHAPTER ELEVEN

The synthesis of 6-alkyl(aryl)-4-hydroxypyrazolo[3,4-d]pyrimidines

Pyrazolo[3,4-d]pyrimidines of the type (102), substituted in the 6-position with an alkyl or aryl group were prepared in a manner similar to the foregoing compounds, by reaction of the reagents α-cyano-β-ethoxy-N-benzoylacrylamide (351) and α-cyano-β-ethoxy-N-acety lacrylamide (292) with hydrazine and substituted hydrazines.

The benzoyl reagent (351) was prepared by a sequence of reactions in which benzamide and cyanoacetic acid were heated together in acetic anhydride to give the benzoyl derivative, N-(cyanoacetyl)-benzamide (349), which, upon reaction with triethyl orthoformate in acetic anhydride gave the required reagent α-cyano-β-ethoxy-N-benzoylacrylamide (351).

In a similar manner, acetamide and cyanoacetic acid in acetic anhydride gave the acetyl derivative (350) which in turn with triethyl orthoformate in acetic anhydride gave the acety lacrylamide reagent, α-cyano-β-ethoxy-N-acety lacrylamide (292).

6-Phenyl-4-hydroxypyrazolo[3,4-d]pyrimidines

Treatment of the benzoyl acrylamide (351) with hydrazine hydrate in warm ethanol gave a yellow precipitate overnight, which gave a strong colour with the Bratton Marshall reagents (λ_{max} 535 nm). The precipitate did not absorb in the -CN region of the i.r. spectrum, however, if the original reaction was performed at 0°, the -CN absorption of the acyclic intermediate (352) was
present in the pale yellow precipitate produced. Recrystallisation of this precipitate gave the pyrazole(353) as a crystalline solid m.p. 152 (resolidified then 290). The mass spectrum of (353) showed a mass ion of 230 which corresponded to the mass required for the pyrazole(353) and a further, and much larger peak at 212 which would correspond to the loss of water. It would therefore appear that the pyrazole(353) cyclised easily and rapidly on the probe with the elimination of water, to give the pyrazolopyrimidine(35Ld).

A sample of the crude pyrazole(353) was briefly heated at 150-170. The solid melted and resolidified to yield a material with m.p. 290. Recrystallisation of the fused material gave 6-phenyl-4-hydroxypyrazolo[3,4-d]pyrimidine(354), m.p. 294-296. The structure of the pyrazolo[3,4-d]pyrimidine(354) was confirmed by elemental analysis, mass spectrometry and u.v. (λ max 252 nm). The mass spectrometry showed a strong mass ion at 212 which corresponded to the mass of the required structure(354), with a secondary peak at 135 corresponding to the loss of a phenyl group.

Reaction of the benzoylacrylamide(351) with methylhydrazine in warm ethanol gave a coloured crystalline product, which possessed no significant -CN absorption in the i.r. spectrum, but which gave strong u.v. absorption at λ max 530 nm, when diazotized and coupled with the Bratton Marshall reagents. Recrystallisation of this material gave the pyrazole(356) as colourless needles, m.p. 204 (effervesced, resolidified then 275). The mass spectrum of (356) showed a mass ion at 244 which corresponded to that required for the pyrazole(356). A second peak at 227 suggested
loss of -OH and a strong third peak at 226 suggested cyclisation on the probe to the pyrazolo[3,4-d]pyrimidine (357) with elimination of water.

A sample of (356), when heated to 220° in an oil bath and briefly maintained at this temperature, melted, bubbled, and then resolidified. 6-Phenyl-4-hydroxy-1-methylpyrazolo[3,4-d]pyrimidine (357) was obtained as a crystalline material, m.p. 280°, from this fused material. Elemental analysis of (357) was in agreement with the proposed structure and its mass spectrum showed a very large mass ion peak at 226 which corresponded to the required structure and a secondary peak at 149 which suggested the loss of a phenyl group. (357) absorbed in the u.v. at λmax 271 nm.

The acyclic intermediate (355) was not isolated, nor was any significant CN absorption observed in the original reaction mixture, even when this mixture was maintained at 0°. Rearrangement of the acyclic intermediate (355) to the pyrazole (356) must therefore occur readily, even at low temperature.

Solutions of the benzoylacrylamide (351) and phenylhydrazine, when mixed, gave an immediate yellow precipitate which continued to form overnight. This material, m.p. 136-138° (resolidifies then 268°), possessed no -CN absorption in its i.r. spectrum, but gave strong absorption in the u.v. (λmax 514 nm) after treatment with the Bratton Marshall reagents. If, however, the original reaction was performed below 5°, then the resulting precipitate possessed strong absorption at 2210 cm⁻¹ (-CN), indicating the presence of the acyclic carbamate (358). Recrystallisation of (358) gave the pyrazole (359) as needles identical to the sample prepared above 5°.
A sample of the foregoing pyrazole(359), was maintained at 200° while it melted, bubbled and resolidified. Recrystallisation of this material (which gave no coloured product with the Bratton Marshall reagents) gave 6-phenyl-4-hydroxy-1-phenylpyrazolo[3,4-d]pyrimidine(360), m.p. 275° (λ_max 275 and 231 nm). The mass spectrometry of (360) showed a very strong mass ion at 288 corresponding to the diphenylpyrazolo[3,4-d]pyrimidine(360).

6-Methyl-4-hydroxy[3,4-d]pyrimidines

In a similar manner the acetyl reagent α-cyano-β-ethoxy-N-acety lacrylamide(292) was treated with hydrazine hydrate, methyl hydrazine and phenyl hydrazine in order to produce 6-methyl substituted-4-hydroxy-1-alkyl(aryl)pyrazolo[3,4-d]pyrimidines.

A methanolic solution of the acetyl reagent(292) with hydrazine hydrate gave an immediate pale brown precipitate, m.p. 115°, which possessed no -CN absorption in the i.r., but which gave a strongly coloured derivative with the Bratton Marshall reagents (λ_max 520 nm). Its mass spectrum showed a weak mass ion peak at 168 which corresponded to the acyclic or pyrazole intermediate (361) or (362) respectively and a very strong peak at 150, corresponding to the loss of water, indicating ready cyclisation of the pyrazole(362) to the pyrazolopyrimidine(363) on the probe. No attempt was made to purify this material further.

A small sample of the crude material was heated slowly in an oil bath. It melted at 115°, bubbling occurred and the temperature was maintained at 170-180° until all bubbling had ceased. The product gave 6-methyl-4-hydroxy[3,4-d]pyrimidine(363) as a crystalline material, m.p. > 300°. This sample gave no
coloured derivative with the Bratton Marshall reagents, but absorbed in the u.v. at $\lambda_{\text{max}}$ 259 nm (pH 11) and $\lambda_{\text{max}}$ 252 nm (pH 1). (363) was found to be identical to a sample prepared by the route suggested by Cheng and Robins, from the 5-amino-4-cyanopyrazole (91a).

This route involved the acetylation of 5-amino-4-cyanopyrazole (91a) with acetic anhydride to give the cyanopyrazole (364), which in turn, when treated with hydrogen peroxide in alkaline solution at 70-80° gave, via the pyrazole (365) the desired 6-methyl-4-hydroxypyrazolo[3,4-d]pyrimidine (363) in excellent yield, and identical to the sample prepared above.

The reaction between the acetyl reagent (292) and methyl hydrazine produced, from a dark coloured solution, crystals, m.p. 218°, possessing weak (CN) absorption at $\lambda_{\text{max}}$ 2230 cm$^{-1}$ in the i.r. spectrum. Recrystallisation gave 5-amino-1-methylpyrazole-4-(N-acetyl) carboxamide (367), which gave a coloured product with the Bratton Marshall reagents (u.v. $\lambda_{\text{max}}$ 509 nm). The mass spectrum of (367) showed several strong peaks. The first, a strong peak at $M^+$, 182, corresponded to the required mass for (367) (C$_7$H$_6$N$_2$O$_2$), the others corresponded to an acceptable breakdown pattern.

A small sample of (367) was heated to 240° on an oil bath. It melted at about 225°, bubbled and resolidified. The fused product, on recrystallisation, gave 6-methyl-4-hydroxy-1-methylpyrazolo-[3,4-d]pyrimidine (368), m.p. 281°, the mass spectrum of which showed two peaks only. The first, a massive peak, indicated the mass ion at 164 which corresponded to the required mass of (368) (C$_7$H$_4$N$_2$O), the second, a minor peak at 149, corresponded to the loss of a methyl group.
This sample was found to absorb in the u.v. at $\lambda_{\text{max}}$ 268 nm (pH 11) and $\lambda_{\text{max}}$ 253 nm (pH 1). (368) was found to be identical (m.p., mixed m.p., t.l.c., u.v.) to a sample prepared by the Cheng and Robins route, previously described for the hydrazine analogue, in which 5-amino-4-cyano-1-methylpyrazole (91b) was acetylated with acetic anhydride and the product (369) treated with alkaline hydrogen peroxide to give the pyrazole (370). The pyrazolopyrimidine (368) was obtained by cyclisation of the pyrazole (370).

No attempt was made to isolate the acyclic intermediate, $\alpha$-cyano-$\beta$-methylhydrazino-N-acetylacrylamide (366) which was responsible for the weak (CN) absorption at $\nu_{\text{max}}$ 2230 cm$^{-1}$, found in the original reaction mixture. Recrystallisation of the mixed product of this reaction ensured complete rearrangement to the methylpyrazole (367), which no longer showed any band in its i.r. spectrum corresponding to (CN).

Phenyl hydrazine with the acetyl reagent (292) in methanol gave a dark crystalline precipitate, recrystallisation of which gave 5-amino-1-phenylpyrazole-4-(N-acetyl)acrylamide (371), m.p. 210° (resolidified and then 290-295°). (371) gave a highly coloured derivative when diazotised and coupled with the Bratton Marshall reagents ($\lambda_{\text{max}}$ 527 nm). The mass spectrum of (371) showed several peaks, the mass ion at 244 (strong) corresponded to the required pyrazole (371) ($C_{15}H_{11}N_4O_3$), the others were typical breakdown products of (371).

A small sample of the pyrazole (371), when heated to 230°, melted, bubbled and resolidified to give needles of the pyrazolopyrimidine (372). Recrystallisation gave 6-methyl-4-hydroxy-1-
phenylpyrazolo[3,4-d]pyrimidine (372) as long needles, m.p. 298°.

The compound failed to give a Bratton Marshall test but absorbed in the u.v. at λ_max 275 nm (pH 11) and λ_max 231 nm (pH 1). The mass spectrum of (372) showed a very strong mass ion at 226 which corresponded to the required mass of (372) (C_{16}H_{10}N_{4}O) and a second peak at 211 corresponded to the loss of a methyl group.

It is interesting to note the difference in stability (as illustrated by melting points and the ease of cyclisation) of pyrazoles prepared from the acetyl reagent with the N_1-position substituted by either methyl (367) or phenyl (371) groups and that prepared from hydrazine, leaving the N_1-position free (362). The hydrazine derivative (362) melted at 115° and cyclised to the corresponding pyrazolopyrimidine, at temperatures as low as 140°, the major peak on the mass spectrum of the pyrazole (362), being that of the pyrazolopyrimidine (363), indicating easy cyclisation on the probe.

The methyl (367) and phenyl (371) derivatives, however, melted at 218° and 210° respectively, and required heating to around 240° before appreciable cyclisation occurred. This was illustrated in both cases by the large mass ion in the mass spectra of (367) and (371), corresponding to the pyrazole, and by the size and multiplicity of the breakdown peaks. The peak corresponding to loss of water, indicating cyclisation on the probe to the pyrazolopyrimidine, to be of relatively minor importance in both spectra.

This difference in cyclisation is shown to a less marked degree in the pyrazoles, (353), (356) and (359) when the benzoyl reagent (351) is treated with hydrazine, methylhydrazine and phenyl-hydrazine respectively.
NC·CH₂·CO·OH
+ R·CO·NH₂ → NC·CH₂·CO·NH₂·CO·R

(349) R = Ph
(360) R = Me

(351) R = Ph
(352) R = Me

(361) R = Me, R₁ = H
(362) R = Me, R₁ = Ph
(363) R = Me, R₁ = H
(364) R = Me, R₁ = Ph
(365) R = Me, R₁ = H
(366) R = R₁ = Me
(367) R = R₁ = Me
(368) R = R₁ = Me
(369) R = R₁ = Me
(370) R = R₁ = Me
(371) R = Me, R₁ = Ph
(372) R = R₁ = Me

(355) R = Me, R₁ = H
(356) R = Me, R₁ = Ph
(357) R = Ph, R₁ = Me
(358) R = Ph, R₁ = H
(359) R = Ph, R₁ = H
(360) R = Ph, R₁ = Me

(91a) R = H
(91b) R = Me
CHAPTER TWELVE

The reaction of ethyl N-(α-cyano-γ-ethoxy-N-methylacryloyl)-carbamate with hydrazines.

In order to prepare pyrazolo[3,4-d]pyrimidines of the type (375), with an N5-methyl substituent on the pyrimidine ring, the reagent, ethyl N-(α-cyano-β-ethoxy-N-methylacryloyl)carbamate (67) was prepared. Treatment of (67) with hydrazine or a substituted hydrazine would, by a reaction sequence analogous to that used in the preceding chapters, give the acyclic hydrazinomethylene derivative (373), which would rearrange to the pyrazole (374). (374), with elimination of ethanol would cyclise to the required 5-methylpyrazolo[3,4-d]pyrimidine-4,6-dione (375).

Three routes to the N-methylacryloylcarbamate (67) were attempted, which are illustrated in fig. 23.

Route (1) was directly analogous to the preparation of ethyl N-(α-cyano-γ-ethoxyacryloyl)carbamate (124) with the exception that, ethyl N-methylcarbamate replaced ethyl carbamate in the condensation with cyanoacetic acid to give N-methyl-N-(cyanoacetyl)carbamate (376), which in turn was converted by treatment with triethyl orthoformate into the N-methyl reagent (67).

Route (2) involved N-methylation of ethyl N-(cyanoacetyl)carbamate and treatment of the resulting ethyl N-methyl-N-(cyanoacetyl)-carbamate (376) with triethyl orthoformate as above.

Route (3) involved direct conversion of ethyl N-(α-cyano-β-ethoxyacryloyl)carbamate (124) into the N-methyl derivative (67).
Route (1)

Ethyl N-methylcarbamate was prepared by the method developed by Hartman and Brethren. Methylamine was treated with ethyl chloroformate and aqueous sodium hydroxide. Vacuum distillation of the product gave ethyl N-methylcarbamate in high yield.

The ethyl N-methylcarbamate was treated with a warmed mixture of cyanoacetic acid and acetic anhydride, the mixture being warmed to facilitate formation of the mixed anhydride (377).

Vacuum distillation of the products of this reaction gave two fractions. The first fraction gave ethyl N-methylacylcarbamate (378) formed by the alternative reaction of the mixed anhydride (377) with ethyl N-methylcarbamate. The second fraction was found to be the required ethyl N-methyl-N-(cyanoacetyl)carbamate (376).

It was found that allowing time for the formation of the mixed anhydride (377) greatly increased the proportion of required product in the final reaction mixture.

Treatment of ethyl N-methyl N-(cyanoacetyl)carbamate (376) with acetic anhydride and triethyl orthoformate gave the required N-methyl reagent (67) which was isolated by vacuum distillation.

Route (2)

Ethyl N-(cyanoacetyl)carbamate (379), obtained by condensation of cyanoacetic acid and ethyl carbamate in acetic anhydride, was treated with etherial diazomethane, until gaseous evolution ceased, and the green colouration of diazomethane persisted. Evaporation to an oil and vacuum distillation gave ethyl N-methyl-N-(cyanoacetyl)carbamate (376) in reasonable yield, identical to the sample prepared by route 1.

Conversion of ethyl N-methyl-N-(cyanoacetyl)carbamate (376)
into ethyl N-(α-cyano-β-ethoxy-N-methylacryloyl)carbamate (67)
was a repetition of the method used in route 1.

**Route (3)**

Direct conversion of the solid, and therefore easily purified, ethyl N-(α-cyano-β-ethoxyacryloyl)carbamate (124), into the N-methyl derivative would seem to be the most obvious route. To this end a suspension of ethyl N-(α-cyano-β-ethoxyacryloyl)carbamate in ether was treated with diazomethane solution. The suspended material slowly dissolved. Eventually all reaction ceased and the green colour persisted. The resulting solution on evaporation gave a yellow oil, portions of which were tested with ammonia, methylamine and phenylethylamine. No reaction was detected with any of these compounds, however, the samples of ethyl N-(α-cyano-β-ethoxy-N-methylacryloyl)carbamate (67) prepared by routes (1) and (2) reacted readily with them all.

Mass spectrometry showed a mass ion of 240 for the product of route (3), however, (67) required (C_{10}H_{14}N_{2}O_{4}) a mass ion of 226. It would appear therefore that two moles of diazomethane had reacted with ethyl N-(α-cyano-β-ethoxyacryloyl)carbamate (124), the first methylated the nitrogen and the second added to the unsaturated bond to form the pyrazoline (380) which would decompose, certainly on the mass spectrometry probe, losing nitrogen to afford the cyclopropane derivative (381); (381), having no active methylene group, is unlikely to react with any of the amines used above.

In terms of both convenience and overall yield, it was route (1) which was found to be the more suitable of the two alternative routes to the N-methyl reagent (67).

In an attempt to prepare 1-phenyl-5-methylpyrazolo[3,4-d]-
\[
\begin{align*}
&\text{NC·CH₂·COOH} \\
&+ \quad \text{H₂N·COOEt} \\
&\quad \quad \rightarrow \quad \text{NC·CH₂·CO·N·COOEt} \\
&\quad \quad \quad \quad \quad \quad \text{(379)}
\end{align*}
\]

\[
\begin{align*}
&\text{NC·CH₂·COOH} \\
&+ \quad \text{MeNH·COOEt} \\
&\quad \quad \rightarrow \quad \text{NC·CH₂·CO·N·COOEt} \\
&\quad \quad \quad \quad \quad \quad \text{(376)}
\end{align*}
\]

\[
\begin{align*}
&\text{Me} \\
&\quad \quad \quad \quad \quad \quad \text{(378)}
\end{align*}
\]
pyrimidine-4,6-dione(384), the above N-methyl reagent(67) was allowed to react with phenylhydrazine. It was assumed that a reaction, similar to that involving phenylhydrazine and ethyl N-(α-cyano-β-ethoxyacryloyl)carbamate(124), would occur, in which the acyclic hydrazino derivative, ethyl N-(α-cyano-β-phenylhydrazino-N-methylacryloyl)carbamate(382) would be produced; (382), in turn, would rearrange to give the pyrazole, 5-amino-1-phenylpyrazole-4-(N-methyl-N-ethylcarbonyl)carboxamide(383), which upon elimination of ethanol would form the required 5-methylpyrazolo[3,4-d]pyrimidine (384).

The reaction between the N-methyl reagent(67) and phenylhydrazine at room temperature was found to give a heavy pale yellow precipitate, which gave a strong absorption peak in the i.r. spectrum at $\nu_{\text{max}}$ 2235 cm$^{-1}$(CN). Recrystallisation of this material gave white needles, m.p. 210, which still gave strong i.r. absorption at $\nu_{\text{max}}$ 2235 cm$^{-1}$(CN).

Upon diazotization and treatment with the Bratton Marshall reagents a sample of the white needles gave no coloured product. Furthermore, the u.v. spectrum of the "needles" was found to be rather similar to the spectra obtained from substituted uracil samples, prepared elsewhere in this thesis. A comparison of the u.v. spectrum of this compound with those of 5-ethoxycarbonyl-1-methyluracil(210), 5-carboxy-1-methyluracil(211) and 1-phenyluracil(77) is shown in the tabular form in fig. 24.

It may be seen, from fig. 24, that the u.v. spectrum of the "white needles", showing absorption at $\lambda_{\text{max}}$ 274 and 218 nm, in neutral solution, and $\lambda_{\text{max}}$ 283 and 212 nm at pH10, is characteristic of the uracil ring system. The examples quoted in fig. 24
showing absorption in the ranges \( \lambda_{\text{max}} \) 287-272 nm and 237-213 nm in neutral solution, and \( \lambda_{\text{max}} \) 285-272 and 237-213 nm at pH10.

Elemental analysis of the "white needles" corresponded to a molecular formula of \( \text{C}_{4}\text{H}_{8}\text{N}_{4}\text{O}_{4} \). This analysis would indicate the "white needles" to be either the 5-methylpyrazolo[3,4-d]pyrimidine (384) or the isomeric 5-cyano-3-methyl-1-anilinouracil(386).

In view of the other evidence, however, namely, the presence of strong CN absorption in the i.r. spectrum, even after recrystallisation of the original reaction product; the absence of any coloured material in the Bratton Marshall test, which would have been found if the intermediate pyrazole(383) had been produced during the reaction sequence; and also the u.v. spectrum of the "white needles" being characteristic of the uracil ring system, it is reasonable to propose that the product of the above reaction is the anilinouracil(386).

It is interesting to note that this ring structure (with no \( N_{3} \) substituent) was the required, and expected, product in the original Shaw synthesis of 1-phenylpyrazolo[3,4-d]pyrimidine-4,6-dione(94c).

The reaction between hydrazine hydrate and ethyl N-(\( \alpha \)-cyano-\( \beta \)-ethoxy-N-methylacryloyl)carbamate(67) was found to be highly exothermic, and on standing gave crystals (387), m.p. 140\(^{\circ}\) (resolidified then 220\(^{\circ}\)). The i.r. spectra of both the original yellow crystalline solid(387) and the solid, after briefly heating at 160\(^{\circ}\), (388) indicated strong absorption at \( \nu_{\text{max}} \) 2240 cm\(^{-1}\)(CN) and the mass spectrum showed a mass ion of 166 for the fused sample, which corresponded to the mass of either the 5-cyano-3-methyl-1-aminouracil(387) or the isomeric 5-methylpyrazolo[3,4-d]pyrimidine-
4,6-dione(389). The strong absorption found in the i.r. spectrum of the fused sample at $\nu_{\text{max}}$ 2240 cm$^{-1}$ (CN), however, confirms the product to be 5-cyano-3-methyl-1-aminouracil(388).

The reaction of the N-methyl reagent(67) with methylhydrazine was also exothermic and gave, on cooling, an oil which solidified, m.p. 210°, when scratched. This material absorbed strongly in the i.r. at $\nu_{\text{max}}$ 2238 cm$^{-1}$ (CN). The mass spectrum of this material showed a mass ion of 180, which corresponded to the mass of either 5-cyano-3-methyl-1-methylaminouracil(391) or its isomer, 1,5-dimethylpyrazolo[3,4-d]pyrimidine-4,6-dione(392). In view of the i.r. spectrum of the fused sample, it is assumed to be the cyanouracil (391). The i.r. spectra of a sample of (391) taken before and after heating the sample to 160° were found to be identical. It would therefore appear that the acyclic intermediate ethyl N-(α-cyano-β-methylhydrazino-N-methylacryloyl)carbamate(390), cyclised spontaneously to 5-cyano-3-methyl-1-methylaminouracil(391) in the original reaction mixture.

It is of interest to note the difference in ease of cyclisation of the hydrazine derivative(387), which required heating to 160°, and the phenylhydrazine derivative(385), and methylhydrazine derivative(390), which spontaneously cyclised to the uracils (386) and (391) respectively.

It may be seen from the results obtained in this and the preceding chapter, that the manner of cyclisation of the acyclic hydrazino derivatives of the ethoxyacryloylcarbamate reagent (124) and the ethoxy N-methylacryloylcarbamate reagent (67) are fundamentally different. The hydrazino derivatives (393) of the ethoxyacryloylcarbamate reagent (124) cyclising to the pyrazole
Fig. 24 Comparison of u.v. absorption spectra of 5-cyano-3-methyl-1-anilinouracil (386) and uracils (210), (211) and (77) (λmax values quoted in nm).
(400) and then to the pyrazolopyrimidine(402), whereas the hydrazino derivative(394) of the N-methylacryloylcarbamate(67) cyclises to the uracil(396).

The only difference between the two reagents is the presence of an N-methyl substituent in reagent (67). It is the presence or absence of this N-methyl substituent therefore, which dictates the manner of cyclisation experienced by the acyclic hydrazino derivative.

From the structures of the compounds (393) and (394), it can be seen that where no N-methyl substituent is present, the possibility exists for keto-enol tautomerism to occur at the 1-carbonyl group. This possibility does not exist with the N-methyl substituent in position. A possible consequence of this difference is outlined in fig. 25.

In the case of the N-methylated acyclic compound (394), ring closure would involve a nucleophilic attack by the lone pair of the 3-hydrazino nitrogen onto the relatively positive carbon of the ester carbonyl, giving the quaternary ammonium intermediate (395), which by elimination of ethanol would give the 1-alkyl(aryl) aminouracil (396).

In the case of the unsubstituted acyclic derivative (393), where keto-enol tautomerism is possible, a different mechanism could operate. If tautomerism did occur at the γ-carbonyl, then by hyperconjugation, the relative electropositive nature of the carbon of the ester carbonyl, would be reduced. An alternative electropositive site does exist, on the carbon of the cyano group. Nucleophilic attack by the lone pair of the α-nitrogen of the hydrazino group, on this carbon atom would give the quaternary
ammonium intermediate (397), which by 1,3-proton transfer from the quaternary nitrogen to the cyano nitrogen would give the unstable imine intermediate (398).

Transfer of the $N_1$ proton on the pyrazole skeleton of (398) to the 5-amino position may occur by an intramolecular 1,4-proton shift, in which one of the other pyrazole molecules acted as the base. It is also possible that the proton transfer could be intermolecular, involving a concerted mechanism, in which one molecule, acting as the base, removed the $N_1$ proton from a second molecule. The intermediate (399), thus formed, would in turn deprotonate a third molecule thus forming the 5-aminopyrazole (400).

The second cyclisation in this molecule, which forms the pyrimidine ring, required either high temperature, or base catalysis, and in some cases both. The mechanism for this ring closure would appear to be as shown in fig. 26.

The base catalyst, in this case the ethoxide ion, would deprotonate the 5-amino group of (400), giving ethanol and the intermediate (401). Attack by the deprotonated nitrogen on the carbon of the ester carbonyl, followed by elimination of an ethoxide ion, would give the pyrazolopyrimidine (402).

A comparison of the ease of cyclisation of the pyrazolopyrimidine from (393), and the uracil from (394), shows that both the cyclisation of (394) to the uracil, and (393) to the pyrazole, were readily accomplished. Complete cyclisation required at most recrystallisation conditions, and often occurred spontaneously.

The second cyclisation of (393), to form the pyrimidine ring was in each case found to be more difficult. This is not unexpected however, if, as is suggested above, deprotonation
of the 5-aminogroup on the pyrazole ring, is a necessary prerequisite to cyclisation of the pyrimidine ring, to give the pyrazolopyrimidine ring system.
CHAPTER THIRTEEN

The synthesis of 5-cyano-3-methyl-1-glycopyranosylaminouracils

The synthesis of a number of 1-glycopyranosylaminouracil derivatives, analogous to the 1-aminouracils prepared in chapter twelve, was attempted. To this end the preparation of a variety of glycosylhydrazones was undertaken. Using methods developed by Tipson, and also by Johnson and Kidd, attempts were made to prepare hydrazones of the following sugars: - L-rhamnose; D-galactose; D-ribose; D-xylose; D-glucose; D-arabinose.

The method used involved treatment of the finely powdered sugar with a cold methanolic solution of anhydrous hydrazine. From this solution was obtained an oil, which was dried under vacuum and dissolved in absolute methanol. Dilution with absolute ethanol gave a precipitated oil, which on washing with methanol, gave for example, L-rhamnopyranosylhydrazone (403), as a crystalline material, m.p. 120. By the same method D-xylose- (406), m.p. 110-114; D-arabinose- (408), m.p. 108-113; and D-galactose- (404), m.p. 118-121, pyranosylhydrazones were isolated as white crystalline solids. D-Glucose- (407) and D-ribose (405) hydrazones unfortunately failed to yield crystalline deposits, despite repeated attempts at purification. The use of L-rhamnose (403), D-galactose- (404) and D-ribose- (405) hydrazones to prepare 1-glycopyranosylaminouracils is described below.

L-Rhamnose hydrazone (403) when treated with a solution of the N-methyl reagent (67), slowly dissolved. The solution was heated under reflux for 2 h, during this time a precipitate was formed
which gave needles(410), m.p. 206 (decomp), from water.

Elemental analysis of (410) indicated a molecular formula of $C_{12}H_{16}N_4O$, which would correspond equally well to the uracil or isomeric pyrazolopyrimidine structures. The i.r. spectrum of (410) showed strong absorption at $\nu_{\max} 2230 \text{ cm}^{-1}(\text{CN})$ and the u.v. spectrum showed absorption peaks at $\lambda_{\max} 279$ and 223 nm in neutral solution, $\lambda_{\max} 276$ and 221 nm at pH 1, and $\lambda_{\max} 281$ and 223 nm at pH 10, characteristic of the uracil ring system. Compound (410) was therefore deduced to be the required 5-cyano-3-methyl-1-L-rhamnopyranosylaminouracil.

The original reaction was performed at room temperature, and a material was deposited which absorbed little in the u.v. but which showed strong absorption in the i.r. at $\nu_{\max} 2230 \text{ cm}^{-1}(\text{CN})$. When heated this material melted at 105°, bubbled and resolidified to melt again at 206°. The fused product was found to be identical (m.p., mixed m.p., u.v.) to (410). This material must therefore be the acyclic intermediate ethyl N-(\(\alpha\)-cyano-\(\beta\)-L-rhamnopyranosylhydrazino-N-methylacryloyl)carbamate(409), which under the original reaction conditions cyclised spontaneously to the uracil(410).

Treatment of the viscous oil from the reaction of hydrazine and D-ribose(405) with a solution of the N-methyl reagent(67), required heating under reflux for some 4 h. Most of the hydrazone dissolved and the solution eventually gave a crystalline deposit, m.p. 175° (decomp). The infra-red spectrum of this deposit showed strong absorption at $\nu_{\max} 2242 \text{ cm}^{-1}(\text{CN})$ and the u.v. spectrum gave absorption peaks in the bands, typical of the uracil ring system, at $\lambda_{\max} 283$ and 221 nm at pH 10 and $\lambda_{\max} 275$ and 220 nm in neutral
solution.

In order to obtain a reasonable solution of ribose hydrazone in the original reaction mixture, it was necessary to heat the mixture under reflux. This precluded the isolation of the acyclic ribopyranosylhydrazone intermediate (411).

The mass spectrum of the crystalline deposit showed a mass ion of 298 which corresponded to a molecular formula of C₉H₁₅N₄O₆. This would again fit either the uracil structure or the isomeric pyrazolopyrimidine structure. However, in view of the absorption band, typical of (CN) in the i.r. spectrum of this material and the typical u.v. spectrum obtained, it is reasonable to propose that compound (412) is 5-cyano-3-methyl-1-D-ribopyranosylamino-uracil.

In an exactly analogous manner, D-galactose hydrazone (404), when treated with ethyl N-(α-cyano-β-ethoxy-N-methylacryloyl)-carbamate (67), proved reluctant to dissolve. Boiling under reflux for some 4 h, gave a solution from which a sticky crystalline deposit was eventually obtained, which gave crystals, m.p. 168°, from water. The i.r. spectrum of this material showed strong absorption at $\nu_{\text{max}}$ 2230 cm⁻¹ (CN), and its u.v. spectrum was typical of the uracil ring system, showing absorption at $\lambda_{\text{max}}$ 279 and 228 nm at pH 10, and $\lambda_{\text{max}}$ 274 and 220 nm in neutral solution.

The mass spectrum of this material showed a mass ion of 328, which again could correspond to either the glycosylamino-uracil (C₉H₁₅N₄O₆) or the isomeric glycosylpyrazolopyrimidine ring systems. In view of the spectral evidence however, it is reasonable to propose that the product of this reaction was
5-cyano-3-methyl-1-D-galactopyranosylaminouracil(413).

No attempt was made to isolate the acyclic intermediates in the preparation of either the ribopyranosylaminouracil(412) or the galactopyranosylaminouracil(413), in view of the reaction conditions required by the process.

The u.v. absorption spectra of the 3-methyl-1-glycosylaminouracils, prepared above, are displayed in tabular form in fig. 27. It may be seen that the absorption bands of these compounds $\lambda_{\text{max}}$ 279-274 nm and $\lambda_{\text{max}}$ 223-220 nm in neutral solution, and $\lambda_{\text{max}}$ 283-279 nm and $\lambda_{\text{max}}$ 228-221 nm at pH10, agree favourably with the u.v. spectra assigned to other uracil derivatives, quoted in previous chapters.

The facile cyclisation undergone by the acyclic intermediates formed by treatment of the above glycosylhydrazones with the N-methyl reagent (67), are in agreement with those observed with the aglycone analogues in the previous chapter. The intermediates preferentially cyclising to the uracil ring system, rather than the pyrazolo[3,4-d]pyrimidine ring system, typical of the hydrazino derivatives of the unmethylated reagent(124), as detailed in chapter nine.
Fig. 27 I.r. and u.v. data for 1-glycopyranosylamino-
uracil derivatives of the N-methylacrylamide
reagent(67) ($\nu_{\text{max}}$ values quoted in cm, $\lambda_{\text{max}}$
values quoted in nm).

| N$_1$glycopyranosyl-
<p>| amine | I.r. absorption | U.v. absorption |</p>
<table>
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<tr>
<th>substituent</th>
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<th>pH 10</th>
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<tbody>
<tr>
<td>L-Rhamnose</td>
<td>2230</td>
<td>276 221</td>
<td>279 223</td>
<td>281 223</td>
</tr>
<tr>
<td>D-Ribose</td>
<td>2242</td>
<td>- -</td>
<td>275 220</td>
<td>283 221</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>2230</td>
<td>- -</td>
<td>274 220</td>
<td>279 228</td>
</tr>
</tbody>
</table>
CHAPTER FOURTEEN

The attempted synthesis of 1-glycopyranosylpyrazolo[3,4-d]pyrimidine-4,6-diones

Following the preparation of the 1-alkyl(aryl)pyrazolo[3,4-d]pyrimidine-4,6-diones, earlier in chapter nine, by treatment of the reagent ethyl N-(α-cyano-β-ethoxyacryloyl)carbamate(124) with alkyl(aryl) hydrazines, it was decided to attempt the preparation of the nucleoside analogues, 1-glycopyranosylpyrazolo[3,4-d]pyrimidine-4,6-diones, by the use of a similar reaction sequence. The preparation of the required glycopyranosyl hydrazones, by reaction of the sugar with hydrazine, was described in the previous chapter, with reference to the preparation of 1-glycopyranosylaminouracils, by treatment of the sugar hydrazone with the reagent ethyl N-(α-cyano-β-ethoxy-N-methylacryloyl)carbamate(67).

Much work was done on the reaction between L-rhamnopyranosylhydrazone and the reagent (124). Unfortunately no analytically pure samples were obtained, therefore any conclusions reached regarding these reactions must be speculative. The reaction was performed in a number of different solvent systems, namely, water, acetonitrile, isopropanol, dioxan, dimethylformamide, and nitromethane, and was thought to proceed in three stages. The first, the formation of the acyclic carbamate, ethyl N-(α-cyano-β-L-rhamnopyranosylhydrazinoacryloyl)carbamate(414); the second, the rearrangement of (414) to give the cyclic structure 5-amino-1-L-rhamnopyranosylpyrazole-4-(N-ethoxycarbonyl)carboxamide(415), and the third stage involved cyclisation of (415) with the elimination
of ethanol, to give 1-L-rhamnopyranosylpyrazolo[3,4-d]pyrimidine-4,6-dione(416).

A material thought to contain the acyclic compound (414) was isolated by treatment of a suspension of rhamnopyranosyl-hydrazone(403) with a solution of the acryloylcarbamate reagent (124), in the cold. Over a period of 3 days, the suspended hydrazone dissolved to be replaced by a flocculant white precipitate, m.p. 118-123 (decomp). This precipitate showed strong absorption in the i.r. at 2250 cm⁻¹(CN) and the u.v. spectrum showed absorption in neutral solution at \( \lambda_{\text{max}} 287 \) nm. The u.v. absorption in alkaline solution moved to \( \lambda_{\text{max}} 273 \) nm and acidification gave an absorption peak at \( \lambda_{\text{max}} 277 \) nm. The original neutral absorption was not re-attainable.

Treatment of the precipitate with alkali, followed immediately by neutralisation with Dowex 50W-X8 (H⁺) resin, gave a solution which absorbed in the u.v., in an identical manner to that described above for the alkali treated u.v. sample. The original absorption peak at \( \lambda_{\text{max}} 287 \) nm again proved unattainable.

The solid foam, m.p. 105-120° (decomp), obtained by evaporation, failed to give the i.r. absorption band at 2250 cm⁻¹(CN) found in the original spectrum. Furthermore, when treated with nitrous acid, and coupled with the Bratton Marshall reagents, the foam gave a highly coloured derivative, (\( \lambda_{\text{max}} \) at 510 nm). It would therefore appear that treatment of the precipitated acyclic derivative(414) with alkali, induced spontaneous cyclisation to the pyrazole isomer(415).

The i.r. and u.v. spectra of the precipitate were compared with the spectra obtained from ethyl N-(\( \alpha \)-cyano-\( \beta \)-hydrazino-
acryloyl)carbamate(320), discussed in chapter nine, and found to be significantly different. The precipitate was therefore not the aglycone derivative, produced by hydrolysis of the original rhamnopyranosyl hydrazone. It was therefore assumed that the precipitate contained ethyl N-\(\alpha\)-cyano-\(\beta\)-L-rhamnopyranosylhydrazinoacryloyl)carbamate(414).

A pure sample of this material was not obtained for two reasons. The first being the extreme reluctance of this precipitate to give any crystalline material, despite the use of many different solvents, and the second being the rapidity with which the acyclic carbamate(414) rearranged, on heating, to the pyrazole(415).

An amorphous sample, thought to contain the rhamnopyranosylpyrazole(415) could be obtained much more rapidly, by performing the reaction between the reagent(124) and rhamnose hydrazone(403) at a higher temperature. Heating under reflux in isopropanol rapidly gave a solution, dilution of which with ether gave a gum, which failed to crystallise. A solid foam, obtained on evaporation of the original solution, was found to be identical (i.r. and u.v.) with the material produced by attempted crystallisation of the precipitate(414). The u.v. for both materials showed absorption at \(\lambda_{\text{max}}\) 273 nm at pH10; \(\lambda_{\text{max}}\) 280 nm in neutral solution and \(\lambda_{\text{max}}\) 278 nm at pH1, there being a small downfield alkali shift. The i.r. spectrum showed no band characteristic of (CN).

A non-crystalline sample of (415) was also obtained from the above reaction performed in dioxan at room temperature. A complete solution obtained overnight, precipitated an oil when diluted with ether. When triturated under ether, the oil gave an amorphous
solid, identical to the samples of (415) prepared above.

Similarly, this reaction performed in other solvents, namely, water, acetonitrile, dimethylformamide and nitromethane, at various temperatures, gave samples of the pyrazole identical to (415) which all failed to yield crystalline products. All samples of the pyrazole(415), when treated with the Bratton Marshall reagents, readily gave a highly coloured derivative, absorbing at $\lambda_{\text{max}}$ 510 nm.

Cyclisation of these crude samples of 5-amino-1-L-rhamnopyranosylpyrazole-4-(N-ethoxycarbonyl)carboxamide(415) was undertaken in several different ways.

1) A number of samples of the amorphous powder(415) were heated in an oil bath. Those maintained below 140° were recovered unchanged. A sample heated to 150-160°, melted, effervesced, but failed to resolidify until cooled. The u.v. spectrum of the fused material showed absorption, at $\lambda_{\text{max}}$ 249 nm in neutral solution, and at $\lambda_{\text{max}}$ 247 nm in alkaline solution, much different to the spectrum of the original amorphous sample(415). Samples maintained above 160° behaved in a similar manner to the above but were more seriously degraded. The product of this reaction is assumed to be 1-L-rhamnopyranosylpyrazolo[3,4-d]pyrimidine-4,6-dione(416).

Purification of this material from water failed to give a crystalline product. Evaporation to dryness gave (416) as a solid foam, m.p. 134-140°.

2) The effect of base catalysed cyclisation of the pyrazole(415) using aqueous alkali was followed by u.v. spectra. No change in the spectrum occurred at any temperature below 60°. At temperatures above 60°, an absorption peak, similar to that found in the fused
sample at $\lambda_{\text{max}}$ 247 nm developed and the absorption at $\lambda_{\text{max}}$ 273 nm, characteristic of the pyrazole(415), slowly faded. The time taken for complete transformation of the spectrum varied with temperature. At 60°C the change required 2 h, whereas when the solution was heated under reflux, the process was complete in some 9 min, leaving no trace of the original pyrazole absorption spectrum.

Evaporation and cooling of the neutralised solution failed to give a crystalline deposit; it was therefore evaporated to dryness, giving a solid foam, identical (mixed m.p., i.r., u.v.) to the purified fused sample of (416) obtained in 1).

3) A sample of the pyrazole(415) was heated under reflux in methyl cellosolve. The u.v. spectrum slowly changed to that of the pyrazolopyrimidine(416), the whole process requiring some 4 h. Purification (charcoal) and evaporation failed to yield a crystalline product. Dilution with ether precipitated an oil, which when triturated under ether, failed to crystallise. Evaporation to dryness gave a solid foam identical (i.r. and u.v.) to the other samples of (416).

4) A sample of the original isopropanol reaction solution containing the hydrazone(403) and the reagent(124) was treated with alcoholic sodium ethoxide, in an attempt to induce base catalysed cyclisation to the pyrazolopyrimidine, in one step, and without having to use aqueous alkali. An immediate pale yellow precipitate was formed of the sodium salt of the pyrazole(415). This precipitate did not provide a means of purifying the pyrazole however, as neutralisation of an aqueous solution of the precipitate also failed to give a crystalline product.

All samples of the pyrazolopyrimidine produced above, were
found to give a positive, although much more pale, Bratton Marshall test, similar to the one given by the pyrazole (415) (λ<sub>max</sub> 510 nm).

Extraction of a sample of (416), using a Soxhlet apparatus, with alcohol, eventually gave an amorphous brown residue, washed free of the Bratton Marshall sensitive component, of the sample (416). An aqueous solution of this pale brown amorphous solid was passed down a column of Amberlite I.R. 120 (NH<sub>3</sub>form) resin. The column was eluted with water. A solution, rich in the material absorbing at λ<sub>max</sub> 248 nm was collected and concentrated, however, despite cooling, scratching and dilution with methanol, no crystalline material was isolated.

The mass spectrum of the solid foam obtained from this purified sample of (416) showed various breakdown products which could correspond to base and sugar fragments, but no mass ion. Therefore, in the absence of any concrete analytical evidence, the structures assigned to (414), (415) and (416) must remain speculative.

In a similar but less exhaustive manner, the reactions of the reagent ethyl N-(α-cyano-β-ethoxyacryloyl)carbamate (124) with the hydrazones of glucose (407), xylose (406) and arabinose (408) were investigated.

The gum (407) produced by the reaction between hydrazine and D-glucose, was treated with a solution of the acryloyl carbamate (124) in isopropanol. Heating under reflux eventually gave a solution which showed u.v. absorption similar to the spectrum of the rhamnopyranosylpyrazole (415). The spectrum showed absorption at λ<sub>max</sub> 283 and 218 nm in neutral solution; at λ<sub>max</sub> 281 and 218 nm at pH 1 and at λ<sub>max</sub> 276 and 224 nm in alkaline solution.
Evaporation to dryness, gave a solid foam m.p. 150-156°, a small sample of which, when diazotised and coupled with the Bratton-Marshall reagents, gave a coloured derivative (λ_{max} 521 nm).

An alkaline solution of the solid foam, assumed to contain 5-amino-1-D-glucopyranosylpyrazole-4-(ethoxycarbonyl)carboxamide (418) was heated under reflux. The cyclisation reaction was followed by u.v. spectra and a change in the position of the absorption peaks was noted from λ_{max} 276 nm to λ_{max} 248 nm, analogous to the spectral change observed during base catalysed cyclisation of (415) above. Despite repeated efforts, no crystalline material was obtained from the neutralised solution. Evaporation gave a solid foam, m.p. 163-167°, thought to be 1-D-glucopyranosyl-
pyrazolo[3,4-d]pyrimidine-4,6-dione(419).

No serious attempt was made to isolate the acyclic intermediate, ethyl N-(α-cyano-β-D-glucopyranosylhydrazinoacryloyl)carbamate(417), as a solid from the original reaction solution. It was assumed, and the u.v. and Bratton Marshall evidence supported this assumption, that cyclisation to the pyrazole(418) had occurred spontaneously under reaction conditions. A small sample of the reaction mixture prior to heating, was retained however, and the i.r. spectrum of the solid, obtained by room temperature evaporation of the solvent, indicated strong absorption at ν_{max} 2210 cm⁻¹(CN) and showed the isolated solid, assumed to be the acyclic intermediate(417), to be different to the starting material.

An aqueous solution of the cyclised foam(419) was placed on a column of Amberlite 120 (NH₃ resin) and eluted with water. Fractions rich in the material, assumed to be the glucopyrazolopyrimidine, absorbing in the u.v. at λ_{max} 248 nm in neutral solution, and at
\( \lambda_{\text{max}} 249 \text{ nm} \) in alkaline solution were isolated. However, no crystalline material was recovered from the solution. Evaporation gave a solid foam, m.p. 165-168.

Treatment of xylopyranosylhydrazone(406), with the acryloyl-carbamate(124) in boiling isopropanol and again in alcohol, gave a heavy precipitate of an aglycone derivative. The glycosyl derivative was isolated from the mother liquors, as the sodium salt, by treatment with sodium ethoxide. The neutralised, aqueous solution of the sodium salt failed to give crystals. Evaporation gave a solid foam(421), m.p. 140-150, which, when diazotised with nitrous acid and coupled with the Bratton Marshall reagents, gave a highly coloured derivative, absorbing at \( \lambda_{\text{max}} 518 \text{ nm} \).

Evaporation of the original mother liquors also gave a yellow solid foam, m.p. 140-150, which was found to be very similar (m.p., u.v. and Bratton Marshall test) to the foam (421) obtained by precipitation of the sodium salt. Recrystallisation attempts failed to give a crystalline material, and t.l.c. of both foam samples indicated at least four components to the mixture.

The u.v. spectrum of (421) showed absorption peaks at \( \lambda_{\text{max}} 281 \) and 218 nm in neutral solution, and at \( \lambda_{\text{max}} 273 \text{ nm} \) in alkali, which fitted into the basic u.v. absorption pattern attributed to the substituted pyrazolo ring structures prepared earlier.

A small sample of the original reaction solution, prior to heating, was set aside. U.v. and i.r. spectra of this solution indicated absorption at \( \lambda_{\text{max}} 287 \text{ nm} \) and \( \nu_{\text{max}} 2260 \text{ cm}^{-1} \text{(CN)} \) respectively. When heated, the band in the i.r. spectrum at 2260 \text{ cm}^{-1} \text{(CN)} disappeared, and the u.v. absorption moved to that of the supposed pyrazole (421) (\( \lambda_{\text{max}} 281 \text{ nm} \)).
From the above spectral data it would appear that the solid foam(421), contained the pyrazole 5-amino-1-D-xylopyranosylpyrazole-4-(ethoxycarbonyl)carboxamide, and that rearrangement of the acyclic intermediate ethyl N-(α-cyano-1-D-xylopyranosylhydrazinoacryloyl)-carbamate(420) occurred in situ, under reaction conditions. No attempt was made to cyclise the pyrazole(421).

The reaction of the arabinose hydrazone(408) and the acryloylcarbamate(124) in dimethyl sulphoxide, eventually gave a solution which showed weak absorption at vmax 2275 cm\(^{-1}\)(CN) in its i.r. spectrum. After warming, no band was found in the i.r. characteristic of (CN). The u.v. spectrum showed absorption at \(\lambda_{\text{max}}\) 281 and 220 nm in neutral solution and at \(\lambda_{\text{max}}\) 277 and 220 nm in alkali.

Dilution with ether precipitated an oil which failed to crystallise. Evaporation gave 5-amino-1-D-arabinopyranosylpyrazole-4-(ethoxycarbonyl)carbamate(422) as a solid foam, which, with the Bratton Marshall reagents, gave a coloured derivative with u.v. absorption at \(\lambda_{\text{max}}\) 525 nm. No crystalline sample of (422) was obtained for elemental analysis, despite repeated attempts at recrystallisation of this foam. This reaction, when repeated using isopropanol as solvent, gave identical results.

The spectral data quoted in fig. 28 for the compounds (414) to (422), when compared to the u.v. absorption spectra, in fig. 22, of the pyrazolopyrimidines prepared in chapter nine, would appear to support the assigned structures.

Acyclic u.v. absorption of the aglycone derivatives occurred at \(\lambda_{\text{max}}\) 282-278 nm; whereas the acyclic glycosyl derivatives absorbed at \(\lambda_{\text{max}}\) 287 nm. Absorption of the aglycone pyrazole ring occurred at \(\lambda_{\text{max}}\) 264-261 nm, as compared to \(\lambda_{\text{max}}\) 277-273 nm for
the glycosyl derivative. The aglycone pyrazolopyrimidines absorbed strongly in the range $\lambda_{\text{max}}$ 248-243 nm (with secondary absorption at around $\lambda_{\text{max}}$ 268 nm), the glycosyl analogue absorbed at $\lambda_{\text{max}}$ 249-247 nm.

In the absence of pure crystalline samples of the compounds (414)-(422), suitable for further analysis however, the evidence for the assignment of the structures shown for the products of the above reactions is not conclusive.
Fig. 28  I.r. and u.v. data for the glycopyranosylhydrazino
derivatives of ethyl N-(\(\alpha\)-ethoxycarbonyl-\(\beta\)-ethc::,\)
acryloyl)carbamate(124) (\(\lambda_{\text{max}}\) values quoted in cm\(^{-1}\);
\(\lambda_{\text{max}}\) values quoted in nm).

<table>
<thead>
<tr>
<th>Glycopyranosyl hydrazone</th>
<th>I.r. absorption</th>
<th>Bratton Marshall</th>
<th>U.v. absorption pH 1 pH 7 pH 10</th>
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<tr>
<td>D-Rhamnose</td>
<td>A 2250</td>
<td>P 510 277 280 273</td>
<td>PP 249 247</td>
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<td>P 510 277 280 273</td>
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<tr>
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<td>P 521 281 283 276</td>
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<td>D-Arabinose</td>
<td>A 2275</td>
<td>P 525 281 277 220</td>
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A - Acyclic intermediate
P - Pyrazole intermediate
PP - Pyrazolo[3,4-d]pyrimidine
Experimental details relating to the synthesis, or attempted synthesis, of uracils contained in chapters four to eight of the discussion.
GENERAL

Evaporations were carried out with a Buchi rotary evaporator under a water-pump vacuum with flask temperature ≤ 50°. Thin layer chromatograms were run on cellulose (Whatman CC 41) or silica-gel coated glass plates (20 x 10 cm) in the system n-butanol-acetic acid-water (12:3:5) and spots were detected by u.v. illumination or with modified Bratton-Marshall spray reagents. U.v. absorption spectra were measured with a Unican SP 800 spectrophotometer, i.r. spectra with a Perkin-Elmer 157 spectrophotometer, and mass spectra with an A.E.I. MS 902 spectrometer.

Ethyl Hydrogen malonate

Diethyl malonate (400g, 2.5mol) dried over drierite was taken up in absolute ethanol (1600ml), alcoholic potassium hydroxide (140g in 1600ml) was added with stirring over 1h, during which time a white precipitate was formed. The suspension was stirred for a further 3h, allowed to stand overnight and subsequently heated to boiling and the hot solution was filtered to remove the insoluble dipotassium salt. Potassium hydrogen malonate crystallised from the solution on cooling, was filtered, ether washed and dried in vacuo. Concentration of the mother liquors gave a second crop of crystals, total yield 350g (82%).

To a chilled solution of the potassium salt (100g, 0.625mol) in water (60ml) in an ice bath, was slowly added with stirring concentrated hydrochloric acid (55ml). The mixture was extracted with ether (4 x 50ml), the extract being dried over sodium sulphate and vacuum distilled. The liquid residue was dried in vacuo at 2.0mm for 3h, yield 79g (96%). The material was of sufficient purity to continue to the next stage without further purification.
Ethyl N-ethoxycarbonylacetylcarbamate (204)

Ethyl hydrogen malonate (13.2 g, 0.1 mol) and acetic anhydride (15.0 g, 0.15 mol) were warmed together at 75° for 15 min. Ethyl carbamate (8.9 g, 0.1 mol) was added and the solution maintained at 75° for 3 h. Evaporation to half volume and cooling gave the acyl carbamate (204) as a crystalline solid, which crystallised from ethyl acetate or petroleum (b.p. 80-100°) as prisms, m.p. 59° (16.2 g, 80%). (Found: C, 47.3; H, 6.45; N, 6.9; C₁₆H₁₃N₂O₅ requires: C, 47.3; H, 6.4; N, 6.9%).

Ethyl N-(α-ethoxycarbonyl-β-ethoxyacryloyl)carbamate (205)

Triethyl orthoformate (350 g, 2.3 mol) and acetic anhydride (450 ml, 4.5 mol) were warmed together at 75° for 10 min, the acyl carbamate (204) (469 g, 2.3 mol) was then added. The solution was boiled under reflux for 1 h and cooled to give the carbamate (205) (230 g, 39%) as needles (from ethyl acetate), m.p. 159°. (Found: C, 51.1; H, 6.6; N, 5.4; M, 259; C₁₅H₁₅N₂O₅ requires: C, 50.96; H, 6.56; N, 5.4%; M, 259).

Ethyl N-(α-ethoxycarbonyl-β-aminoacryloyl)carbamate (206)

Ammonia was passed into a solution of the carbamate (205) (3 g, 11.5 mol) in methanol at 5-10 for 30 min to give a white precipitate of the acyclic compound (206) (2.8 g), m.p. 164° (resolidifies). (Found: M⁺, 230; Calc. for C₉H₈N₂O₅; M, 230); m/e, 184 (M - C₂H₅OH).

5-Ethoxycarbonyluracil (207)

To a solution of the acyclic compound (206) (0.92 g, 4 mmol) in ethanol (20 ml) was added ethanolic sodium ethoxide (3 ml, 4.35 mmol). The solution was warmed on a steam bath for 5 min and cooled to give a white gel which dissolved after adjustment of the pH to 6.8,
with M hydrochloric acid (6 ml). 5-Ethoxycarbonyluracil (207) separated out as an amorphous solid which crystallised from ethanol-ethyl acetate as plates, m.p. 184. (Found: C, 45.6; H, 4.46; N, 15.4; M, 184. C$_7$H$_9$N$_2$O$_4$ requires: C, 45.7; H, 4.35; N, 15.2%; M, 184).

5-Carboxyuracil (208)

a) The carbamate (206) (1.45 g, 5.6 mmol) was dissolved in aqueous sodium hydroxide solution (20 ml, 70 mmol) and kept at room temperature for 30 min. The sodium salt of 5-carboxyuracil separated as an amorphous solid (1.0 g, 95%), which was dissolved in hot water (30 ml). The pH was adjusted to 6.0 with 2 M hydrochloric acid to give a white precipitate of 5-carboxyuracil, m.p. 225° (from water). (Found: M$,^+$ 156; Calc. for C$_5$H$_4$N$_2$O$_4$; M, 156); m/e, 112(M - CO$_2$)$^+$. 

b) The above experiment was repeated with the alkaline solution of the ester boiling under reflux. Aliquots (1 ml) were taken at timed intervals, made up to 25 ml with distilled water and titrated against standard sulphuric acid using phenolphthalein as indicator. The titration studies (fig 10) indicated that complete cyclisation and hydrolysis had occurred within the first minute or so.

c) A small amount of 5-ethoxycarbonyluracil in aqueous M sodium hydroxide was kept at room temperature for 1 h. Titration studies (fig. 9) performed as above showed this to be the minimum time required for complete hydrolysis to have occurred. The pH was adjusted to 6.0 with 2 M hydrochloric acid, to give 5-carboxyuracil (208) identical with the samples from a) and b), m.p. 223°. (Found: C, 38.8; H, 2.44; N, 17.8; M$,^+$ 156; C$_5$H$_4$N$_2$O$_4$ requires: C, 38.5; H, 2.56; N, 17.95%; M, 156); m/e 112(M - CO$_2$)$^+$. 
Decarboxylation of 5-carboxyuracil (208)

a) Direct heating: A small sample of 5-carboxyuracil was heated using an oil bath at various temperatures. No change was noticed in the sample below temperatures of 280°. The sample was maintained at 280-300° for 30 min after which it proved to be identical (i.r., t.l.c., mixed m.p.) with an authentic sample of uracil.

b) Quinoline and copper powder: A solution of 5-carboxyuracil (0.18 g) in quinoline (3 ml) was boiled under reflux with activated copper powder (0.03 g) for 1 h, and cooled to room temperature. To the mixture was added 2M sodium hydroxide solution (4 ml) and water (2 ml). The whole was extracted with ether (4 X 10 ml) and the aqueous phase acidified with 5M hydrochloric acid and extracted with ethyl acetate (3 X 10 ml). On evaporation the ethyl acetate extract gave a yellow solid identical to an authentic sample of uracil. It was found that for this method to be effective a minimum temperature of 200-210° was required.

c) Heat with basic copper carbonate: A small amount of the carboxyuracil was intimately mixed with an equal volume of basic copper carbonate and heated. No change was observed in the carboxyuracil until a temperature of 275° was reached. Above this temperature decarboxylation readily occurred and a product, identical to an authentic sample of uracil was obtained.

d) Heat in N,N-dimethylaniline: A solution of 5-carboxyuracil (1 g) in N,N-dimethylaniline (8 ml) was heated. Gaseous evolution commenced at 160° and was vigorous at 190°. The solution was maintained at 190° for 1 h and the cool solution gave crystals of a material (0.82 g) which was identical (i.r., t.l.c., mixed m.p.)
with an authentic sample of uracil.

e) Heat in N-ethylmorpholine: A small amount of 5-carboxy-uracil was heated in N-ethylmorpholine to 120-130° over 20 min. Carbon dioxide was evolved and the solid dissolved. Uracil was precipitated from the cooled solution with ether and purified by dissolution in M sodium hydroxide solution and neutralisation with 2 M sulphuric acid; the product was identical (i.r., t.l.c., mixed m.p.) with an authentic sample of uracil.

Activated copper powder: To a solution of hydrated copper sulphate crystals (CuSO₄·5H₂O) (100 g) in water (350 ml) at room temperature was added zinc dust with stirring until the solution became colourless. The precipitated copper powder was decanted and water washed. Dilute hydrochloric acid solution (5 %) was added to remove the excess zinc and the whole was agitated until the evolution of hydrogen gas ceased. The copper powder was filtered, water washed and kept moist in an air tight container until required.

Ethyl N-(α-ethoxycarbonyl-β-methylaminoacryloyl)carbamate (209)

Methyamine (2.5 ml of a 25 % ethanolic solution, 20 mmol) was added to a solution of ethyl N-(α-ethoxycarbonyl-β-ethoxyacryloyl)-carbamate (205) (5.2 g, 20 mmol) in ethyl acetate (3 ml); the solution was kept at room temperature for 12 h and evaporated to dryness to give ethyl N-(α-ethoxycarbonyl-β-methylaminoacryloyl)-carbamate (209) as a solid foam, which crystallised as plates, m.p. 120° from ethyl acetate-petroleum (b.p. 60-80). (Found: C, 49.2; H, 6.6; N, 11.5; M, 244; C<sub>11</sub>H<sub>11</sub>N<sub>2</sub>O<sub>5</sub> requires: C, 49.18; H, 6.56; N, 11.4 %; M, 244); m/e 198 (M - EtOH).  

5-Ethoxycarbonyl-1-methyluracil (210)

To a solution of the carbamate (209) (1 g, 4 mmol) in ethanol
(10 ml) was added a 2% solution of sodium ethoxide in ethanol (8 ml, 7 mmol); the solution was warmed on a steam bath for 15 min, neutralised with 2 M hydrochloric acid, evaporated to half volume, and cooled, to yield 5-ethoxycarbonyl-1-methyluracil as a crystalline solid, m.p. 237° (from ethyl acetate). (Found: M+, 198; Calc. for C9H9N2O3: M, 198).

5-Carboxy-1-methyluracil(211)

a) From 5-ethoxycarbonyl-1-methyluracil(210) :- The ester (210) (1.98 g, 10 mmol) was dissolved in aqueous sodium hydroxide solution (20 ml containing 1.6 g, 70 mmol) and left at room temperature. Aliquots (1 ml) were taken at timed intervals and titrated against 0.1 M sulphuric acid using phenolphthalein as indicator. Hydrolysis was found to be complete in 1 h, fig. 13. The solution was initially neutralised with acetic acid, however, this yielded a white solid, m.p. >360°, which proved to be the sodium salt of the required acid.

In later experiments the solution was either neutralised with a strong (mineral) acid, namely 2 M hydrochloric acid, which gave a white precipitate, m.p. 269, which in turn gave 1-methyl-5-carboxyuracil(211) as needles from water; or the alkaline solution was neutralised with Dowex 50W-X8 resin (H+) to give a white precipitate, m.p. 269-270°, identical to that obtained by using hydrochloric acid. (Found: C, 42.5; H, 3.4; N, 16.9; M+, 170; C9H9N2O3 requires: C, 42.3; H, 3.53; N, 16.47%; M, 170); m/e 126(M - CO2)+.

b) From the carbamate(209) :- The carbamate (1 g, 4.6 mmol) was dissolved in aqueous sodium hydroxide solution (10 ml containing 0.45 g, 19.5 mmol) at room temperature. After 10 min the pH was adjusted to 6.0 with Dowex 50W-X8 resin (H+) and the solution
was evaporated to half volume to give a crystalline precipitate of 5-carboxy-1-methyluracil, m.p. 270, identical (i.r., t.l.c.) with the foregoing material.

1-Methyluracil(212)

From the 5-carboxy-1-methyluracil(211) by :-

a) Direct heating :- A finely divided solid sample of 5-carboxy-1-methyluracil (1 g) was heated progressively until decarboxylation occurred. At temperatures up to 220° no change was noted either in the physical form of the sample or in its u.v. spectrum. At 240° the sample began to darken, at 260-270 the sample melted and effervescence occurred and the u.v. spectrum changed considerably from absorption at λmax 279 and 222 nm to absorption at λmax 272 and 213 nm (both in neutral solution). The material gave crystals from nitromethane identical (i.r., u.v., mixed m.p.) to an authentic sample of 1-methyluracil.

b) By heating with quinoline and copper :- A suspension of 5-carboxy-1-methyluracil (1 g) and activated copper powder (0.1 g) in quinoline (10 ml) was heated to 200°. Slight effervescence was noted which became more pronounced when the temperature was raised to 210-220°. The suspension was maintained at this temperature for 1 h. Recrystallisation of the suspended material from nitromethane gave crystals of 1-methyluracil identical with the foregoing material.

c) By heating with N,N-dimethylaniline :- A small amount of 5-carboxy-1-methyluracil was heated in N,N-dimethylaniline to 160-180° over 30 min; gas was evolved and the solid dissolved. 1-Methyluracil was precipitated from the cooled solution with ether and gave crystals from nitromethane, identical with the foregoing material.
d) **By heating with N-ethylmorpholine**: A small amount of 5-carboxy-1-methyluracil was heated in N-ethylmorpholine to 120-130° over 20 min; gas was evolved and the solid dissolved. 1-Methyluracil was precipitated from the cooled solution with ether and gave crystals from nitromethane; the product was identical (i.r., u.v., mass. spec., t.l.c., m.p., mixed m.p.) with an authentic sample of 1-methyluracil.

**Ethyl N-(α-ethoxycarbonyl-β-anilinoacryloyl)carbamate (213)**

To a solution of ethyl N-(α-ethoxycarbonyl-β-ethoxyacryloyl)carbamate (1.3 g, 5 mmol) in benzene (15 ml) was added aniline (0.5 g, 5.5 mmol). The solution was warmed on a water bath for 15 min and, on cooling, gave a crystalline precipitate of ethyl N-(α-ethoxycarbonyl-β-anilinoacryloyl)carbamate (213) (1.4 g, 92%), m.p. 113° (resolidified).

**5-Ethoxycarbonyl-1-phenyluracil (217)**

The ethoxycarbonylacrylamide (213) (1 g) was dissolved in ethanol (10 ml) and to this solution was added 2% alcoholic sodium ethoxide (8 ml, 7 mmol). The mixture was warmed on a steam bath for 15 min and the pH adjusted to 6.8 with 2 M hydrochloric acid to give a flocculent white precipitate of 5-ethoxycarbonyl-1-phenyluracil (217) which crystallised from alcohol as needles, m.p. 294° (decomp).

(Found: M⁺ 260; Calc. for C₁₃H₁₀N₂O₆: M⁺ 260).

**5-Carboxy-1-phenyluracil (218)**

a) **From the carbamate (213)**: Ethyl N-(α-ethoxycarbonyl-β-anilinoacryloyl)carbamate (213) (1.3 g, 4.2 mmol) was dissolved, by warming in aqueous sodium hydroxide (15 ml, 11 mmol) and the pH of the solution was adjusted to 6.5 with 2 M hydrochloric acid to give a white precipitate of 5-carboxy-1-phenyluracil (218) (1.0 g, 100%)
which crystallised from water as needles, m.p. 284°. (Found: M, 232, Calc. for C₉H₈N₂O₄; M, 232; m/e, 188(M - CO₂).

b) From the ester uracil(217) :- A small amount of 5-ethoxy-
carbonyl-1-phenyluracil(217) was dissolved in aqueous sodium hydroxide (10 ml, 7.5 mmol). The solution was kept at room temperature for 1 h then neutralised with 2 M hydrochloric acid to give 5-carboxy-
1-phenyluracil(218) identical (i.r., t.l.c.) with the foregoing sample.

1-Phenyluracil(77)

A suspension of the carboxyuracil(218) (1.0 g, 4.3 mmol) in
N-ethylmorpholine (5 ml) was heated at 120° for 15 min, during which
time rapid evolution of gas was observed. 1-Phenyluracil(77) was
filtered off and recrystallised from ethyl acetate as plates, m.p. 321°
(0.7 g, 86 %). (Found: C, 64.0; H, 4.1; N, 14.8; M, 188; C₉H₈N₂O₄
requires : C, 63.83; H, 4.26; N, 14.89 %; M, 188).

Ethyl N-(α-ethoxycarbonyl-β-benzylaminoacryloyl)carbamate(214)

To a solution of ethyl N-(α-ethoxycarbonyl-β-ethoxyacryloyl)-
carbamate(205) (5.2 g, 20 mmol) in ethanol (30 ml) was added
benzylamine (2.2 ml, 30 mmol) whereupon the solution immediately
attained a strong yellow colouration. Evaporation of this solution
to low volume gave an oil which solidified over several days.
Recrystallisation from ethyl acetate gave ethyl N-(α-ethoxycarbonyl-
β-benzylaminoacryloyl)carbamate(214) as needles, m.p. 320°. (Found: M, 320; Calc. for C₆H₈N₂O₅; M, 320; m/e 274 (M - EtOH).

5-Ethoxycarbonyl-1-benzyluracil(219)

To a solution of the foregoing acyclic carbamate(214) (6.4 g)
in absolute ethanol (30 ml) was added a solution of sodium ethoxide
in ethanol (0.69 g in 20 ml) and the solution was set aside for 1 h
at room temperature. The solution was neutralised with M hydrochloric acid, evaporated to half volume and cooled. 5-Ethoxycarbonyl-1-benzyluracil(219) was obtained as an amorphous white solid which gave crystals, m.p. 264°, from nitromethane. (Found: C, 61.3; H, 5.21; N, 10.2; M, 274; \(\text{C}_9\text{H}_7\text{N}_2\text{O}\) requires: C, 61.1; H, 5.11; N, 10.1%; M, 274).  

**Ethyl N-\(\alpha\)-ethoxycarbonyl-\(\beta\)-phenylethlaminoacryloyl)carbamate(215)**

To a solution of ethyl N-\(\alpha\)-ethoxycarbonyl-\(\beta\)-ethoxyacryloyl)-carbamate(205) (5.2 g, 20 mmol) in ethanol (25 ml) was added phenylethylamine (3 ml, 30 mmol), and the green solution was kept at room temperature for 1 h and then diluted with water (50 ml). An oil was precipitated which, upon cooling and scratching, gave ethyl N-\(\alpha\)-ethoxycarbonyl-\(\beta\)-phenylethlaminoacryloyl)carbamate(215) as an amorphous solid, which gave crystals from nitromethane, m.p. 89°.  

**5-Ethoxycarbonyl-1-phenylethyluracil(220)**

To a solution of ethyl N-\(\alpha\)-ethoxycarbonyl-\(\beta\)-ethoxyacryloyl)-carbamate(205) (5.2 g, 20 mmol) in ethanol (25 ml) was added phenylethylamine (3 ml, 30 mmol). To the resulting green solution was added a solution of sodium ethoxide in ethanol (1 g sodium in 15 ml). An immediate white precipitate was obtained, thought to be the sodium salt of the acyclic carbamate(215a), which redissolved on standing at room temperature overnight. M Hydrochloric acid (50 ml) was added and 5-ethoxycarbonyl-1-phenylethyluracil(220) was obtained as an amorphous solid which gave crystals, m.p. 172°, from nitromethane. (Found: M, 288; Calc. for \(\text{C}_{15}\text{H}_{16}\text{N}_2\text{O}\): M, 288).  

**5-Carboxy-1-phenylethyluracil(221)**

a) From the acyclic carbamate(215) :- Ethyl N-\(\alpha\)-ethoxycarbonyl-\(\beta\)-phenylethlaminoacryloyl)carbamate(215) (8.0 g) was dissolved with
warming in 2 M sodium hydroxide solution (30 ml). The pH of this solution was adjusted to 6.8 with 2 M hydrochloric acid and the resulting white precipitate (5.9 g) of 5-carboxy-1-phenylethyluracil (221) was filtered, washed with water, ethanol and diethyl ether. The carboxyuracil(221) was obtained as a crystalline material from ethyl acetate-petroleum ether (b.p. 60-80), m.p. 189° (Found: M⁺, 260; Calc. for C₁₉H₁₈N₂O₄: M, 260); m/e 216 (M - CO₂). 

b) From the 5-ester uracil(220) :- A solution of 5-ethoxycarbonyl-1-phenyluracil(220) (0.34 g) in aqueous sodium hydroxide (0.195 g in 20 ml) was refluxed and aliquots (1 ml) were taken at intervals and titrated against standard hydrochloric acid using phenolphthalein as indicator (fig. 11). Complete hydrolysis required 2 h after which the solution was neutralised with 2 M hydrochloric acid and the precipitated 5-carboxy-1-phenylethyluracil(221) purified as in the foregoing experiment. The product was found to be identical to that prepared from the acyclic carbamate(215).

Ethyl N-(α-ethoxycarbonyl-β-furfurylaminoacryloyl)carbamate(216)

To a solution of ethyl N-(α-ethoxycarbonyl-β-ethoxycarbonyl)-carbamate(205) (5.4 g, 20 mmol) in ethanol (25 ml) was added furfurylamine (2.4 g, 30 mmol). The green solution was diluted with water (100 ml), and ethyl N-(α-ethoxycarbonyl-β-furfurylaminoacryloyl)carbamate(216) was precipitated as a sticky solid, which crystallised when scratched and gave crystals from ethyl acetate (5.9 g), m.p. 137°.

5-Ethoxycarbonyl-1-furfuryluracil(222)

To a solution of the ethoxycarbonylcarbamate(205) (5.4 g, 20 mmol) in alcohol (25 ml) was added furfurylamine (2.4 g). To the resulting green solution was added a solution of sodium in ethanol
(1g in 20 ml). An immediate precipitate of the linear sodium salt was obtained, which redissolved on standing. The solution was neutralised with M hydrochloric acid (50 ml), cooled, and gave on scratching, white plates of 5-ethoxycarbonyl-1-furfuryluracil (222) which gave white plates, m.p. 205°, from ethanol (3.3 g).

(Found: M, 264; Calc. for C$_9$H$_8$N$_2$O$_5$: M, 264).

5-Carboxy-1-furfuryluracil (223)

a) From the carbamate (216):- To a solution of ethyl N-(α-ethoxycarbonyl-β-ethoxyacryloyl)carbamate (205) (5.4 g, 20 mmol) in ethanol (25 ml) was added furfurylamine (2.4 g). A sticky precipitate of the acyclic carbamate (216) was obtained on dilution with water (100 ml) which was recovered and dissolved in M sodium hydroxide (70 ml) with warming. The pH of the resulting green solution was adjusted to 6.8 with M hydrochloric acid (70 ml) and 5-carboxy-1-furfuryluracil (223) separated as an amorphous white solid (4.3 g) which gave crystals, m.p. 189°, from water, that caused evolution of carbon dioxide from sodium bicarbonate solution. (Found: C, 50.94; H, 3.50; N, 11.92; M, 236; C$_9$H$_8$N$_2$O$_5$ requires: C, 50.8; H, 3.39; N, 11.86 %) m/e 192 (M - CO$_2$).+

b) From 5-ethoxycarbonyl-1-furfuryluracil (222):- A solution of the uracil ester (222) (0.66 g) in aqueous sodium hydroxide (0.4 g, 20 ml) was boiled under reflux. Aliquots were taken (1 ml) at intervals and titrated against standard 0.05 M sulphuric acid using phenolphthalein as indicator (fig. 12). Complete hydrolysis required 1 h after which time the pH of the solution was adjusted to 6.8 with 2 M hydrochloric acid. 5-Carboxy-1-furfuryluracil (223) separated out as an amorphous solid which gave crystals from water (0.4 g) identical (mixed m.p., i.r., u.v. and m.s.) with the product
of the foregoing experiment.

D-Xylopyranosylamine(224)

Ammonia gas was bubbled into methanol (500 ml) at 0 °C until a saturated solution was obtained (1 h). The solution was allowed to warm to room temperature, and D-xylose (170 g) was added. The mixture was magnetically stirred until all solid had dissolved (3 h). D-Xylopyranosylamine was obtained as a crystalline solid over 6 days, m.p. 128-129 °C (160 g).

D-Glycopyranosylamine(225)

D-Glucose (10 × 20 g) was added to a stirred, cooled solution of ammonia in methanol (200 ml). During the addition ammonia was bubbled through the mixture. D-Glycopyranosylamine(225) was obtained as a crystalline material (170 g), m.p. 125-127 °C, over 10 days.

L-Rhamnopyranosylamine(226)

L-Rhamnose monohydrate (25 g) dissolved rapidly in ice-cold ammonia-saturated methanol (50 ml) with ammonia gas bubbling through. The solution was kept at 4 °C for 2 weeks, crystallisation occurred after 1 week; L-rhamnopyranosylamine(226) (19 g, 77%) was filtered off, washed with methanol and recrystallised from dry methanol; m.p. 114-116 °C.

D-Ribopyranosylamine(227)

D-Ribose (10 × 20 g) was added to a stirred, cooled solution of ammonia in methanol (200 ml). During the addition ammonia was bubbled through the mixture, which was then transferred to a heavy gauge polythene bag and stored at 4 °C. After 2 weeks the product (180 g, 90%), m.p. 128-129 °C, was broken up, collected, washed with methanol and dried in vacuo.

3,5-O-Isopropylidene-D-xylofuranosylamine toluene-p-sulphonate(228)

Powdered D-xylopyranosylamine(224) (40.8 g, 0.275 mmol) was
added to a vigorously stirred solution of dry toluene-p-sulphonic acid monohydrate (105 g, 0.55 mol) in 2,2-dimethoxypropane (229 g, 2.2 mol) and dry acetone (1000 ml). The solution was stirred for 15 h, then evaporated to about half its volume. Crystallisation began after 5 min and 3,5-O-isopropylidene-D-xylofuranosylamine toluene-p-sulphonate (228), m.p. 121° (decomp) was obtained in high yield.

D-Mannopyranosylamine monohydrate

D-Mannose (100 g) and ammonium chloride (0.5 g) were dissolved with stirring in ice-cold ammonia-saturated methanol (100 ml) with ammonia gas bubbling through. The solution was kept at 4° for 1 day, then seeded to give crystals of D-mannopyranosylamine monohydrate which were filtered off, washed with methanol, and dried. The product (110 g, 99%) had m.p. 92-93°.

2,3:5,6-Di-O-isopropylidene-D-mannofuranosylamine toluene-p-sulphonate (229)

D-Mannopyranosylamine (49.1 g, 0.275 mol) was added to a stirred solution of dry toluene-p-sulphonic acid monohydrate (105 g, 0.55 mol) in 2,2-dimethoxypropane (286 ml, 2.2 mol) and dry acetone (1000 ml). The solution was stirred for 12 h, then evaporated to about half volume, and dry ether (about 500 ml) added to render the solution almost turbid, the mixture was set aside at 4°. 2,3:5,6-Di-O-isopropylidene-D-mannofuranosylamine toluene-p-sulphonate (229) separated out as a crystalline solid after 20 min, which was collected, ether washed, and dried to give needles (32 g, 66%); m.p. 132-134° (decomp).

2,3-O-Isopropylidene-D-ribofuranosylamine toluene-p-sulphonate (280)

Powdered D-ribopyranosylamine (40.8 g, 0.275 mol) was added to a vigorously stirred solution of dry toluene-p-sulphonic acid
monohydrate (105 g, 0.55 mol) in 2,2-dimethoxypropane (286 ml, 2.2 mol) and dry acetone (1000 ml). The solution was stirred for 15 h, then evaporated to half its volume, and dry ether (about 500 ml) was added to render the solution almost turbid. Crystallisation began within 20 min and the mixture was kept overnight at 0. The product (280) was then collected, washed with ether (dry) and dried in vacuo to give needles (78 g, 80%), m.p. 128-129° (decomp).

2,3-O-Isopropylidene-L-rhamnofuranosylamine toluene-p-sulphonate

L-Rhamnopyranosylamine (20 g, 0.12 mol) was added to a stirred solution of dry toluene-p-sulphonic acid monohydrate (32 g, 0.17 mol) in dry acetone (100 ml) and 2,2-dimethoxypropane (100 ml, 0.82 mol) to give immediately, a clear solution. Dry ether was added to incipient turbidity and the solution was stored at 0 overnight. A thick oil separated which rapidly crystallised when stirred vigorously with the supernatant for 4-5 h. The product was collected, washed with dry acetone then dry ether, and dried in vacuo to give the furanosylamine salt (14.5 g, 31%), m.p. 143° (decomp).

5,6-O-Isopropylidene-D-glucofuranosylamine toluene-p-sulphonate

Dry, finely powdered, D-glucopyranosylamine (100 g, 0.56 mol) was stirred into a solution of dry toluene-p-sulphonic acid monohydrate (160 g, 0.84 mol) in dry acetone (500 ml, 6.8 mol) and 2,2-dimethoxypropane (500 ml, 4.1 mol) in a stoppered flask. After 35 min the precipitate (60 g, 41%) was collected, washed three times with dry acetone and twice with dry ether, and dried in vacuo to give needles, m.p. 122-123° (decomp). If the precipitated product was not removed from the reaction it redissolved in about 1 h.
Ethyl N-(α-ethoxycarbonyl-β-D-xylopyranosylaminoacryloyl)carbamate (233)

A mixture of D-xylopyranosylamine (224) (0.7 g) and ethyl N-(α-ethoxycarbonyl-β-ethoxyacryloyl)carbamate (205) (10.4 g) was dissolved by shaking in D.M.S.O. (30 ml). The solution was maintained at room temperature for 30 min and then diluted with ether (about 500 ml) until the first sign of turbidity was noticed. The solution, on standing for 16 h, gave a crystalline solid (233) (9 g) which was ether washed and dried in vacuo. The acrylamide gave needles, m.p. 147-149°, from isopropanol. (Found: M, 362; Calc. for C_{14}H_{12}N_2O_9: M, 362); m/e 317 (M - EtO), 316 (M - EtOH), 183 (base fragment C_7H_{12}O_4), 133 (sugar fragment C_5H_9O_4). (U.v. \lambda_{max} (pH 4) 272 nm; \lambda_{max} (pH 7) 284 nm; \lambda_{max} (pH 10) 273 nm).

5-Ethoxy-β-D-xylopyranosyluracil (234)

A mixture of the glycosylamine (224) (6.0 g, 0.04 mol) and ethyl N-(α-ethoxycarbonyl-β-ethoxyacryloyl)carbamate (205) (10.4 g, 0.04 mol), was shaken in D.M.S.O. (30 ml). After 5 min a solution was obtained which was kept at room temperature for 1 h. M Sodium ethoxide solution in ethanol-D.M.S.O. (40 ml) was added and the solution was kept at room temperature overnight.

A precipitate of the sodium salt of the uracil (234) (12 g) was obtained on dilution with ethanol, which was washed with ethanol-ether and dissolved in water (80 ml). Neutralisation of the aqueous solution with Dowex 50W-X8 (H^+) resin and cooling at 0° gave a jelly which slowly crystallised over several days to give fine needles of 5-ethoxycarbonyl-1-β-D-xylopyranosyluracil (234), m.p. 172-173°. (Found: C, 45.4; H, 5.31; N, 8.59; M, 316; C_{14}H_{12}N_2O_9 requires: C, 45.6; H, 5.06; N, 8.86 %; M, 316). (U.v. \lambda_{max} (pH 7), 274 and 220 nm, \lambda_{max} (pH 10), 274 and 218 nm).
**5-Carboxy-1-β-D-xylopyranosyluracil (235)**

The acyclic glycopyranosylcarbamate (233) (6.0 g) was dissolved with stirring in aqueous sodium hydroxide (2.1 g in 20 ml). The solution was kept at room temperature for 15 min and then the pH was adjusted to 6.0 with Dowex 50W-X8 (H⁺) resin. 5-Carboxy-1-β-D-xylopyranosyluracil (235) separated immediately, the solution was therefore warmed to 60 and the resin removed. The solution gave (235) on cooling as a crystalline solid (3.9 g), m.p. 188° (decomp), which gave short needles, m.p. 192° (decomp), from water. (Found: M, 156; Calc. for C₁₀H₁₂N₂O₅: M, 288); m/e, 156 (base fragment, C₅H₃N₂O⁺), 133 (sugar fragment, C₅H₄O⁺).

**1-β-D-Xylopyranosyluracil (236)**

a) **Using xylene solution of toluene-p-sulphonic acid**: The uracil carboxylic acid (235) (0.1 g) was suspended in a solution of toluene-p-sulphonic acid in xylene (0.1 g in 7 ml) and the mixture was heated to 140° for 2 h. The suspension was recovered by filtration and was found to be identical (i.r., u.v., m.p., mixed m.p.) with the original acid (235). Evaporation of the mother liquors gave only toluene-p-sulphonic acid.

b) **Heating in N,N-dimethylaniline**: A suspension of the carboxyuracil (235) (0.2 g) in dimethylaniline (1 ml) was heated over 10 min to 180°. At this temperature gaseous evolution occurred however extensive degradation of the solid also occurred, which rendered the subsequent work up and purification of the glycosyluracil impossible.

c) **Heating in N-ethylmorpholine**: The carboxyuracil (235) (0.05 g) was suspended in N-ethylmorpholine (3 ml). The mixture was heated over 10 min to 135°, carbon dioxide being liberated above 125°.
Gaseous evolution ceased after 15min, the solid was collected, washed with ether and gave needles of 1-β-D-xylopyranosyluracil (236) from aqueous ethanol, m.p. 215-220. (Found: M, 133; Calc. for C₉H₈N₂O₆: M, 244); m/e, 133 (sugar fragment, C₅H₆O₅); 111, (base fragment, C₄H₃N₂O₂), (u.v. λ max (pH 7), 265, 206 nm; λ max (pH 10), 264, 206 nm).

Ethyl N-(α-ethoxycarbonyl-β-D-glucopyranosylaminoacryloyl) carbamate (237)

A solution of D-glucopyranosylamine (225) (1.8 g) and ethyl N-(α-ethoxycarbonyl-β-ethoxyacryloyl) carbamate (205) (2.6 g) in ethanol (30 ml) was obtained by warming to near boiling point for 3-4 min. On cooling a gelatinous precipitate was obtained which was filtered, ether washed and allowed to dry to an amorphous powder, which gave crystals from alcohol/water, m.p. 153.

5-Ethoxycarbonyl-1-β-D-glucopyranosyluracil (238)

A solution of D-glucopyranosylamine (225) (3.6 g) and ethyl N-(α-ethoxycarbonyl-β-ethoxyacryloyl) carbamate (205) (5.2 g) in D.M.S.O. (15 ml) was maintained at room temperature for 24 h, and then diluted with petroleum ether (b.p. 40-60) (30 ml).

A solution of sodium ethoxide (2 g sodium per 100 ml) (23 ml) was added and the whole heated to 60° for 10 min. On cooling the sodium salt of the ester uracil separated as a gel. The dry sodium salt was dissolved in water (25 ml) and the pH adjusted to 6 with Dowex 50W-X8 (H⁺) resin. The solution on cooling slowly gave 5-ethoxycarbonyl-1-β-D-glucopyranosyluracil (238) as plates (2.1 g), m.p. 244°. (Found: M, 346; Calc. for C₁₃H₁₂N₂O₆: M, 346); m/e, 183 (base fragment, C₇H₅N₂O₂); 163 (sugar fragment, C₆H₇O₅), (u.v. λ max (pH 7), 272, 217 nm; λ max (pH 10), 275, 212 nm).
5-Carboxy-1-β-D-glucopyranosyluracil (239)

Ethyl N-(ω-ethoxycarbonyl-1-β-D-glucopyranosylaminoacryloyl)-carbamate (205) (2 g) was dissolved in 2M aqueous sodium hydroxide solution by warming gently for 5 min. The pH was adjusted to 5.8 with 2M hydrochloric acid and the solution reduced in volume to one third. Cooling for 2 days at 0-5° gave crystals which effervesced with aqueous sodium bicarbonate solution. 5-Carboxy-1-β-D-glucopyranosyluracil (239) separated from a water-alcohol mixture as plates, m.p. 225° (Found: M, 318; Calc. for C_{n}H_{m}N_{o}O_{p}; M, 318) m/e, 274 (M-CO_{2}); 163 (sugar fragment C_{n}H_{m}O_{p}); 155(base fragment C_{n}H_{m}N_{o}) (u.v. λ_{max}(pH7) 268, 213 nm; λ_{max}(pH10), 263, 206 nm).

1-β-D-Glucopyranosyluracil (240)

a) Heating in D.M.S.O: A suspension of 5-carboxy-1-β-D-glucopyranosyluracil (239) (0.5 g) in D.M.S.O. (15 ml) was heated to boiling point. Extensive degradation occurred as the mixture darkened. Samples were taken at various intervals and u.v. analysis of these samples indicated no change had occurred. Decarboxylation would be expected to be accompanied by a down shift of the two absorption peaks at pH7 (λ_{max} 268, 213 nm).

b) Heating in N,N-dimethylaniline: A small sample of the uracil carboxylic acid (239) was heated in N,N-dimethylaniline (15 ml) at 180° for 10 min, and carbon dioxide was evolved. U.v. evidence suggested new absorption peaks at λ_{max} 265 and 207 nm. However, the dark residue resisted all attempts at purification.

c) Heating in quinoline with copper powder: To a solution of the uracil carboxylic acid (239) (1 g) in quinoline (5 ml) was added copper powder (0.15 g) (freshly prepared by a displacement reaction between zinc dust and copper sulphate solution). The mixture was heated on an oil bath and gaseous evolution commenced at 180°. Carbon
dioxide gas was evolved, detected with calcium hydroxide solution, and a u.v. analysis similar to that of b) was obtained. The final mixture however, was again too badly degraded to successfully purify.

d) Heating in N-ethylmorpholine:—A suspension of the uracil carboxylic acid(239) in N-ethylmorpholine, when heated to 130°, gave bubbles of carbon dioxide. The suspension was maintained at this temperature for 15 min, after which time gaseous evolution had ceased. During this time, u.v. analysis showed a rapid and progressive shift from \( \lambda_{\text{max}} \) 268 and 213 nm to \( \lambda_{\text{max}} \) 264 and 205 nm. 1-ß-D-Glucopyranosyluracil(240) was recovered as an amorphous powder which gave needles from aqueous ethanol, m.p. 238°.

(Found: C, 43.4; H, 4.93; N, 10.1; M, 274; \( \text{C}_9 \text{H}_{17} \text{N}_2 \text{O} \), requires: C, 43.8; H, 5.11; N, 10.22 %, M, 274); m/e, 163(sugar fragment, \( \text{C}_6 \text{H}_{10} \text{O}_5 \)), \( \text{u.v.} \lambda_{\text{max}} \) (pH 7), 264, 205 nm; \( \lambda_{\text{max}} \) (pH 10), 263, 204 nm).

**Ethyl N-(α-ethoxycarbonyl-ß-L-rhamnopyranosylaminoacryloyl)-carbamate(241)**

A solution of L-rhamnopyranosylamine(226) (3.3 g) and ethyl-N-(α-ethoxycarbonyl-ß-ethoxyacryloyl)carbamate(205) (5.2 g) in D.M.S.O. (30 ml) was maintained at room temperature overnight. The solution was diluted with alcohol (15 ml) and sufficient ether (150 ml) to produce the first sign of turbidity. Ethyl N-(α-ethoxycarbonyl-ß-L-rhamnopyranosylaminoacryloyl)carbamate(241) separated as a white crystalline material (6.5 g) which gave platelets from alcohol/water, m.p. 180° (decomp). (Found: M, 376; Calc. for \( \text{C}_9 \text{H}_{16} \text{N}_2 \text{O}_9 \); M, 376); m/e, 147(sugar fragment \( \text{C}_6 \text{H}_{10} \text{O}_5 \)).
**5-Carboxy-1-α-L-rhamnopyranosyluracil (242)**

Ethyl N-(α-ethoxycarbonyl-β-L-rhamnopyranosylaminoacryloyl)-carbamate (241) (3.76 g, 0.01 mol) was stirred in aqueous sodium hydroxide solution (1.5 g in 20 ml) at room temperature, the solid dissolved after 2 min. After 15 min the solution was neutralised to pH 6.5 with M sulphuric acid. On dilution with alcohol (10+20 ml) the cooled solution gave a gel, which proved to be sodium sulphate, in near quantitative yield. On cooling, the mother liquors gave 5-carboxy-1-α-L-rhamnopyranosyluracil (242) as a gel, which slowly crystallised (1.9 g) over three to four days, and effervesced with aqueous sodium bicarbonate solution, m. p. 248. (Found: M, 302, Calc. for C₁₁H₁₄N₂O₉: M, 302) m/e 258(M - CO₂); 147(sugar fragment C₆H₇O₄); 111(base fragment, C₄H₃N₂O₂) (u. v. λ max (pH 7) 269, 213 nm; λ max (pH 10), 264, 205 nm).

**1-α-L-Rhamnopyranosyluracil (243)**

A suspension of 5-carboxy-1-α-L-rhamnopyranosyluracil (242) (0.5 g) in N-ethylmorpholine (10 ml) was heated to 120°. Carbon dioxide evolution commenced and became quite vigorous. The suspension was maintained at 130-135° for 1 h, after which time all decarboxylation had ceased. 1-α-L-Rhamnopyranosyluracil (243) was recovered as an amorphous powder which gave white platelets (0.35 g) from aqueous ethanol, m. p. 258°. (Found: C, 46.4; H, 5.6; N, 10.8; M, 258. C₁₁H₁₄N₂O₂ requires: C, 46.5; H, 5.43; N, 10.85%, M 258) (u. v. λ max (pH 7), 265 205 nm; λ max (pH 10) 263, 205 nm).

**5-Ethoxycarbonyl-1-α-D-ribopyranosyluracil (245)**

A solution of D-ribopyranosylamine (227) (3.0 g) and ethyl N-(α-ethoxycarbonyl-β-ethoxyacryloyl)carbamate (205) (5.4 g) in D.M.S.O (20 ml) was left to stand at room temperature over a weekend. On
dilution with petroleum ether (b.p. 40-60) (50 ml) and alcohol (10 ml) the acyclic carbamate(244) was precipitated as an oil. This oil was taken up in methanol (20 ml) and a solution of sodium methoxide in methanol (2 g sodium/100 ml) (23 ml) was added. After 2 h the solution was evaporated to give an oil which with alcohol gave the sodium salt of the ribopyranosyluracil ester(246a) as a white solid (10.7 g). A solution of this sodium salt in water was adjusted to pH 6.0 with Dowex 50W-X8 (H⁺) resin. On standing overnight the cooled solution gave 5-ethoxycarbonyl-1-β-D-ribopyranosyluracil(245) as a gel (1.9 g) which slowly crystallised as needles.

Concentration of the mother liquors gave a second gel identical to the first, m.p. 188° (decomp). (Found: C, 44.2; H, 4.91; N, 9.04; M, 316; C₁₁H₁₄N₁O₄ requires: C, 44.3; H, 5.06; N, 8.86%; M, 316) (u.v. λmax(pH7) 274, 220 nm; λmax(pH10) 274, 219 nm).

5-Cyano-1-β-D-ribopyranosyluracil (248)

A solution of D-ribopyranosylamine(227) (1.41 g) in water (30 ml) was added to one of ethyl N-(α-cyano-β-ethoxyacryloyl)carbamate (205) (2 g) in warm ethanol (30 ml) and kept at room temperature for 1 h. The solution was evaporated to dryness and the resulting gum dissolved in water (10 ml) and treated with a solution of basic lead acetate. There was no immediate precipitate but when the solution was warmed on the steambath for a few minutes and then cooled, a thick solid precipitate was obtained. The lead salt was filtered off, washed with a little ice water, suspended in ethanol, and decomposed with hydrogen sulphide. After removal of lead sulphide the colourless solution was evaporated to a small volume and separated from ethanol as needles m.p. 231°. (Absorption λmax. at 274 and 215 nm). In 24 h at 20 the substance consumed 2.1 mols of
sodium metaperiodate and liberated 1.23 mols of formic acid.

Ethyl N-(α-cyano-β-ethoxyacryloyl)carbamate (124)

Ethyl N-cyanoacetylcarbamate (Conrad and Schulze, Loc. cit) (3.12 g), triethyl orthoformate (2.96 g) and acetic anhydride (5 ml) were boiled under reflux for 1 h, the crystalline product (124) separated on cooling, was washed with light petroleum, and crystallised from benzene as needles (3.2 g), m.p. 120°.

5-Cyano-1-(2,3-O-isopropylidene-α-D-ribofuranosyl-)uracils (251) and (250)

a) A solution of 2,3-O-isopropylideneribofuranosylamine toluene-
-p-sulphonate (230) (10.8 g, 30 mmol) in methanol (100 ml) was treated with methanolic 2 M sodium methoxide (15 ml). Ethyl N-(α-
cyano-β-ethoxyacryloyl)carbamate (124) (6.35 g, 30 mmol) was added, and the solution left for 1 h, then treated with more 2 M sodium methoxide (15 ml) and set aside overnight. The solution was evaporated and a solution of the residue in water (50 ml) was acidified with acetic acid at 0°. The precipitate was collected and washed with water; t.l.c. showed this to be a mixture. Fractional crystallisation from a large volume of ethanol gave the uracil β-nucleoside (250) as white needles, m.p. 226°. The mother-liquors were evaporated to a small volume and cooled to yield a sample enriched in material corresponding to the lower of the two t.l.c. spots. Recrystallisation from a large volume of 95 % ethanol gave as a first fraction, pure uracil α-nucleoside (251) as white needles, m.p. 267-268°.

b) To a solution of 2,3-O-isopropylideneribofuranosylamine toluene-p-sulphonate (0.369 g, 1.0 mmol) in saturated aqueous sodium hydrogen carbonate (10 ml) was added ethyl N-(α-cyano-β-
ethoxyacryloyl)carbamate (124) (0.21 g, 1.0 mmol) and the mixture
was warmed gently to give a green solution, which was left at room
temperature overnight. The solution was cooled in ice and the pH
was adjusted to 7 with 2M hydrochloric acid to yield a white
precipitate of 5-cyano-1-(2',3'-O-isopropylidene-β-D-ribofuranosyl)-
uracil (251) (0.052 g, 15.5%), which crystallised from 95% ethanol
as needles, m.p. and mixed m.p. 269 (with the material prepared in a)).
The mother liquor was further acidified to pH3 to yield the β-
isomer (250) (0.025 g, 7.8%) which crystallised from 95% ethanol
as needles, m.p. and mixed m.p. 216.

5-Cyano-1-β-D-ribofuranosyluracil (249)

A suspension of the isopropylidene-β-nucleoside (250) (4.9 g, 16 mmol) in aqueous 30% acetic acid (50 ml) was heated on a boiling
water bath for 3 h then evaporated to dryness. The residue was
repeatedly evaporated with water to remove the acetic acid. When a
solution of the remaining foam in ethanol (20 ml) was boiled for a
few min, 5-cyano-β-D-ribofuranosyluracil (250) (3.4 g, 80%) separated
as needles, m.p. and mixed m.p. 185 (decomp). In 24 h at 25° the
compound had consumed 1.1 mol equivalent of 0.10 M sodium periodate
without the formation of formic acid.

5-Ethoxycarbonyl-1-(2',3'-O-isopropylidene-β-D-ribofuranosyl)uracil (260)

a) To a solution of 2,3-O-isopropylideneribofuranosylamine toluene-
p-sulphonate (230) (10.8 g 30 mmol) in methanol (30 ml) was added
a solution of sodium ethoxide (30 mmol) in ethanol (34.5 ml) followed
by the carbamate (205) (9.0 g, 35 mmol). The solution was kept at
room temperature for 2 h then warmed on a steambath for 10 min.
More ethanolic sodium ethoxide (34.5 ml, 30 mmol) was added and the
solution was left at room temperature overnight, heated on a water
bath for 0.5 h, and evaporated to a foam.
a) The foam was dissolved in water (40 ml), the pH adjusted to 5.8 with 2 M sulphuric acid and the solution was filtered, concentrated, and seeded with a crystal. The product, 5-ethoxycarbonyl-1-(2',3'-O-isopropylidene-β-D-ribofuranosyl)uracil(260) (3.4 g, 32%) crystallised over several days and was recrystallised from water to give short needles, m.p. 196. °C (Found: C, 50.1; H, 5.75; N, 7.6; C₁₅H₁₅N₂O₅ requires: C, 50.5; H, 5.62; N, 7.86%) (u.v. λ max (pH 7) 274 and 219 nm).

b) Repeating the above experiment but using sodium methoxide in methanol as the base, gave a lower yield of the ester uracil(260) identical to that obtained above, together with a low melting material (m.p. 55) which gave needles from ethyl acetate and was found to be identical to an authentic sample of ethyl N-ethoxy-carbonylacetamidocarbamate.

c) Experiment a) using ethanol and sodium ethoxide, or t-butanol and sodium t-butoxide, gave products identical to the ester(260) but in much lower yield.

d) Experiment a) using cyanomethane as solvent and triethylamine as the base indicated, by u.v. analysis, that the acyclic carbamate(259) had formed (u.v. λ max 284 nm), however, heating with excess triethylamine failed to cyclise the acyclic compound. 1 mole equivalent of sodium ethoxide in ethanol was added and the ester uracil(260) was isolated as above.

5-Carboxy-1-(2',3'-O-isopropylidene-β-D-ribofuranosyl)uracil(261)

a) To a solution of 2,3-O-isopropylideneribofuranosylamine toluene-p-sulphonate (2.4 g, 6.7 mmol) in methanol (10 ml) were added ethanolic sodium ethoxide (6.7 ml, 6.7 mmol) and the carbamate(205)(1.7 g, 6.6 mmol). The solution was kept at room temperature for 1 h and
evaporated to dryness. The residue was dissolved in water (15 ml) and sodium hydroxide (0.8 g, 35 mmol) was added; the solution was kept at room temperature overnight then neutralised with Dowex 50W-X8 resin (H⁺) and evaporated to dryness. The residue crystallised from nitromethane-petroleum to give the acid (261), m.p. 186-192° (decomp). (Found: C, 47.9; H, 4.5; N, 8.75; M⁺; 328; C₁₇H₁₇N₁₀O₃ requires: C, 47.56; H, 4.88; N, 8.54%, M, 328) m/e 313 (M - Me), 284(M - CO₂)⁺ 270(M - Me₂CO), 254(M - Me₃CO₂)⁺, (u.v. λ max (pH7) 272, 216 nm).

b) A solution of the ester (260) (1.6 g, 4.5 mmol) and sodium hydroxide (0.72 g, 31 mmol) in water (18 ml) was heated on a water bath for 2h, then left at room temperature overnight. The pH was adjusted to 6.0 with Dowex 50W-X8 resin (H⁺) and the solution was evaporated to a foam which crystallised from nitromethane-petroleum ether (b.p. 40-60) to give the acid (261) (1.1 g, 75%), identical with the sample from a).

Uridine (158)

A suspension of the acid (261) (2 g, 6.1 mmol) in N-ethylmorpholine (15 ml) was heated to 130-135° for about 1 h (until evolution of gas ceased). The suspension was filtered and the decarboxylated derivative (262) (1.7 g) was washed with ether and boiled under reflux in aqueous 30% acetic acid (20 ml) for 3 h. The resulting solution was evaporated to dryness, acetic acid was removed by evaporation with toluene (3 X 15 ml). The resulting dry foam was shaken with absolute ethanol, the suspension was filtered and ether was added to the filtrate to precipitate uridine (0.9 g, 60%), identical with an authentic sample (i.r., u.v., m.p., m.s. and t.l.c.) (Found: C, 44.3; H, 4.85; N, 11.6; M, 244; C₉H₉N₁₀O₃ requires: C, 44.3; H, 4.92; N, 11.48%; M, 244) (u.v. isopropylideneuridine(262) λ max (pH7) 276, 214 nm; uridine(158) λ max (pH7) 262 nm).
To a solution of 3,5-O-isopropylidene-D-xylofuranosylamine toluene-\(\beta\)-sulphonate (14.4 g, 40 mmol) in acetonitrile (50 ml) was added a solution of sodium ethoxide in ethanol (1.84 g sodium in 25 ml, 80 mmol) and ethyl N-(\(\alpha\)-ethoxycarbonyl-\(\beta\)-ethoxyacryloyl)-carbamate(205) (10.4 g, 45 mmol). The solution turned a green colour and sodium toluene-\(\beta\)-sulphonate was precipitated and removed. The solution was warmed on a steam bath for about 20 min and diluted with ether (50 ml) giving a sticky precipitate of the sodium salt of the required product, which was collected and dissolved in water (20 ml). This solution was adjusted to pH 6.2 with Dowex 50W-X8 resin (H\(^{+}\)) and evaporated to an oil which when evaporated with alcohol (3 \(\times\) 15 ml) gave a pale brown solid foam, which was easily ground to a fine powder (5.8 g, 40\%). The original mother liquors gave a second crude sample, similar to that above, when evaporated to dryness with ethanol. The original sample gave 5'-ethoxycarbonyl-1-(3',5'-O-isopropylidene-\(\beta\)-D-xylofuranosyl)uracil(263) as short needles, m.p. 190\(^{\circ}\) (decomp), from water over several days. (Found: C, 50.3; H, 5.7; N, 7.8; M\(^{+}\), 356; C\(_{12}\)H\(_{16}\)N\(_2\)O\(_2\) requires: C, 50.56; H, 5.62; N, 7.87\% M, 356) m/e 341(M - Me\(^{+}\)); 326(M - Me\(_2\)CO\(^{+}\)); 298(M - Me\(_2\)CO\(_2\)O\(^{+}\)); (u.v. \(\lambda\) max (pH7) 273, 220 nm).

5-Carboxy-1-(3',5'-O-isopropylidene-\(\beta\)-D-xylofuranosyl)uracil(264)

A solution of the uracil ester(263) (1 g) in aqueous sodium hydroxide solution (0.45 g, in 10 ml) (3 mole equivalent) was maintained at reflux for 2 h and then neutralised with Dowex 50W-X8 resin (H\(^{+}\)) and evaporated to dryness, to yield a solid foam, m.p. 140\(^{\circ}\) (decomp), which effervesced with aqueous sodium bicarbonate solution. (Found: M\(^{+}\), 328, Calc. for C\(_9\)H\(_{16}\)N\(_2\)O\(_2\): M, 328) m/e 313(M - Me\(^{+}\)); 284(M - CO\(^{+}\)); 270(M - Me\(_2\)CO\(^{+}\)); 226(M - Me\(_2\)CO\(_2\)O\(^{+}\)); (u.v. \(\lambda\) max(pH7))
1-β-D-Xylofuranoslyuracil (266)

A suspension of the acid (264) (1 g, 3 mmol) was heated in N-ethylmorpholine (10 ml). At about 110° the suspension softened and became rather sticky. Gaseous evolution commenced at 128° (confirmed to be carbon dioxide by the limewater test) and the suspension was maintained at 130-135° for about 45 min. During this time the sticky suspension slowly changed into a hard amorphous pale brown solid, and the solution colour darkened. The suspension was removed and added to more N-ethylmorpholine (10 ml) and heated to 130° for a further 30 min until all stickiness had gone from the suspension. The solid was recovered and ether washed (2 X 10 ml), 1-(3',5'-O-isopropylidene-β-D-xylofuranosyl)uracil (265) gave crystals m.p. 248° from ethyl acetate-alcohol.

The uracil (265) was boiled under reflux in 30% aqueous acetic acid (20 ml) for 3 h. The resulting solution was evaporated to dryness, acetic acid was removed by evaporation with water (3 X 15 ml), and the water was removed by evaporating with toluene (3 X 15 ml). The resulting dry foam was shaken with absolute ethanol, the resulting solution gave 1-β-D-xylofuranosyluracil (266) as small needles m.p. 265° (decomp). (Found: C. 44.1; H. 5.1; N, 11.3; M, 244; C₉H₁₀N₂O₆ requires: C, 44.3; H, 4.92; N, 11.48%; M, 244) (u.v. λ max(pH7) 261 nm). (Found for the isopropylideneuracil (265), M⁺ 284; Calc. for C₉H₁₀N₂O₆; M, 284) m/e 269(M - Me); 173(M - uracil); 112(uracil)⁺. (u.v. λ max(pH7) 265, 214 nm).

5-Ethoxycarbonyl-1-(2',3',5',6'-di-O-isopropylidene-α-D-mannofuranosyl)-uracil (268)

To a solution of 2,3:5,6-di-O-isopropylidene-D-mannofuranosyl-
amine toluene-\text{-}p-sulphonate (229) (8.7 g, 20 mmol) in methanol (20 ml) was added 2\% ethanolic sodium ethoxide (23 ml, 20 mmol) and ethyl-N-(\alpha\text{-}ethoxycarbonyl-\beta\text{-}ethoxyacryloyl)carbamate (205) (5.5 g, 25 mmol), and the whole was allowed to stand for 1 h at room temperature. A second mole equivalent of 2\% sodium ethoxide (23 ml) was added and an immediate precipitate was obtained which slowly dissolved overnight. The solution was warmed on a steam bath for 2 h and evaporated to dryness. The resultant solid foam was dissolved in water (20 ml) and this solution was adjusted to pH 5.8 with M sulphuric acid. A white precipitate formed slowly and was collected and recrystallised from aqueous ethanol. 5-Ethoxycarbonyl-1-(2',3':5',6'-di-O-isopropylidene-\alpha\text{-}D-mannofuranosyl)uracil (268) was obtained as short needles, m. p. 210-214° (decomp), (5.5 g, 64\%). (Found: M, 411; Calc. for C_{15}H_{16}N_{2}O_{5}; M, 426) m/e 411 (M - Me$^+$).

5-Carboxy-1-(2',3':5',6'-di-O-isopropylidene-\alpha\text{-}D-mannofuranosyl)uracil (269)

The ester uracil (268) (2.13 g, 5 mmol) was dissolved in an aqueous solution of potassium hydroxide (0.84 g, 15 mmol in 15 ml) and the resulting solution was heated on a water bath for 90 min, neutralised with M sulphuric acid to pH 5.8, cooled and diluted with ethanol (10 ml). A precipitate of potassium sulphate was removed and the solution, when evaporated to dryness, gave a solid foam (1.25 g, 62\%) of the required product, m. p. 242° (decomp). (Found: M$^+$, 398; Calc. for C_{15}H_{16}N_{2}O_{5}; M, 398) m/e 383 (M - Me$^+$); 354 (M - CO$_2$); 340 (M - Me$_2$CO $^+$).

1-\alpha\text{-}D-Mannofuranosyluracil (271)

A suspension of the acid (269) (1 g, 2.5 mmol) in N-ethylmorpholine (10 ml) was heated to 130-135° for about 1 h (until evolution of gas had ceased). The suspension was filtered and the
solid (270) (0.8 g) was washed with ether and boiled under reflux in aqueous 30% acetic acid for 3 h. The resulting solution was evaporated to dryness, acetic acid was removed by evaporating with water (3 × 10 ml), and the water was removed by evaporating with toluene (3 × 15 ml). The resulting dry foam was shaken with absolute ethanol which gave on dilution with ether, 1-α-D-mannofuranosyluracil (271) (0.4 g, 62%) as a crystalline powder, m. p. 253 (decomp). (Found: M, 274; Calc. for C₉H₁₀N₄O₃: M, 274) (u.v. λ max (pH 7) 260 nm)

5-Ethoxycarbonyl-1-(2',3'-O-isopropylidene-β-D-rhamnofuranosyl)uracil (272)

To a solution of 2,3-O-isopropylidene-D-rhamnofuranosylamine-toluene-sulphonate (231) (3 g, 8 mmol) in methanol (15 ml) was added a solution of 2% sodium ethoxide (9.3 ml, 8 mmol) followed by the carbamate (205) (2.4 g, 9.4 mmol). The solution was kept at room temperature for 1 h then a second mole equivalent of sodium ethoxide (9.3 ml) was added, and the solution was set aside overnight, heated on a steam bath for 2 h, and then evaporated to a foam. The foam was dissolved in water (20 ml), the pH was adjusted to 6.0 with M sulphuric acid, and the solution was filtered, concentrated and cooled. The ethoxycarbonyluracil (272) separated as needles, m. p. 186 over several days. (Found: M⁺, 370; Calc. for C₁₅H₂₁N₂O₅ M, 370), m/e 355 (M - Me)⁺; 297 (M - MeC₂O, - Me). 5-Ethoxycarbonyl-1-(5',6'-O-isopropylidene-β-D-glucofuranosyl)uracil (273)

To a solution of 5,6-O-isopropylidene-D-glucofuranosylamine-toluene-sulphonate (232) (8 g, 21 mmol) in methanol (20 ml) was added a solution of 2% ethanolic sodium ethoxide solution (23 ml, 20 mmol) followed by the carbamate (205) (5.5 g, 25 mmol). The solution was kept at room temperature for 1 h. More 2% ethanolic sodium ethoxide (23 ml, 20 mmol) was added and the solution was
heated on a steam bath for 30 min, stood at room temperature overnight, and evaporated to a foam. The foam was dissolved in water (20 ml), the pH was adjusted to 6.5 with $\text{H}_2\text{SO}_4$, and the solution was filtered and concentrated. The ester uracil (273) crystallised over several days, m.p. 236 (decomp), (Found: M, 386$	ext{C}_{10}\text{H}_{12}\text{N}_{2}\text{O}_{3}\text{N}, M$, 386), m/e 371 (M - Me); 328 (M - MeCO).

**Ethyl N-methylcarbamate**

A mixture of 33% aqueous methylamine (186 g, 2 mol) and ether (300 ml) was mechanically stirred in a 2000 ml flask and cooled in an ice/salt bath to below 5. Ethyl chloroformate (217 g, 2 mol) was added slowly and the temperature was maintained below 5. When about half of the ethyl chloroformate had been added a cold solution of sodium hydroxide (80 g, 2 mol) in water (120 ml) was added gradually along with the rest of the chloroformate at such a rate that the final addition of sodium hydroxide solution corresponded to the final addition of the chloroformate. The mixture was allowed to stand for 15 min, and the ether layer separated and was collected. The aqueous layer was extracted with ether (2 X 50 ml) and the combined etherial solutions were treated with potassium carbonate (2 X 4 g). The solution was evaporated and the residual oil distilled under reduced pressure. The distillate collected at 55-60/12mm (182 g, 88%) as a colourless liquid.

**Ethyl N-ethoxycarbonylacetyl-N-methylcarbamate (275)**

Route a) :- A solution of ethyl hydrogen malonate (132 g, 1 mol) in acetic anhydride (200 g) was heated on a steam bath for 10 min to ensure formation of the mixed anhydride. Ethyl N-methylcarbamate (103 g, 1 mol) was added and the solution was heated on a steam bath for 3 h and diluted with water (2000 ml). An oil was precipitated which was extracted with ether (4 X 250 ml), the ethereal
layer being washed with aqueous sodium bicarbonate solution, and water (3 X 200 ml) and dried over anhydrous sodium sulphate.

Evaporation of the ethereal solution gave an oil (185 g) which was distilled under vacuum and gave two fractions, the first boiling at 39-41/0.1 mm and the second distillate, collected at 108/0.1 mm (128 g, 59%), gave ethyl N-ethoxycarbonylacetyl-N-methylcarbamate (275) as a colourless liquid.

**Route b):** To a stirred suspension of ethyl N-ethoxycarbonylacetylcarbamate(204) (20 g) in ether (100 ml) was added a solution of diazomethane in ether. An immediate evolution of nitrogen occurred and the natural diazomethane colour (yellow/green) faded. More diazomethane was added until visible reaction has ceased and a stable green colour persisted. Excess diazomethane was destroyed by the addition of acetic acid, dropwise until no further reaction occurred. This solution on evaporation gave an oil which upon vacuum distillation gave two fractions. The first distillate collected at 60-120/5 mm(Hg) pressure, the second distillate collected at 120-130/5 mm as a colourless oil (10 g, 45%) which was found (i.r.,t.l.c.,u.v.) to be identical to the oil (275) from route a).

**Ethyl N-(α-ethoxycarbonyl-β-ethoxy-N-methylacryloyl)carbamate(276)**

**Route a)(and b):** Triethyl orthoformate (16 g, 0.1 mol) and acetic anhydride (30 ml, 0.3 mol) were warmed together at 75° for 10 min, and the N-methylcarbamate(275) (21.7 g, 0.1 mol) was added. The solution was heated at 85° for 3 h and fractionally distilled under vacuum. The first distillate contained most of the volatiles and was collected at 28/0.4 mm(Hg) pressure. Ethyl N-(α-ethoxycarbonyl-β-ethoxy-N-methylacryloyl)carbamate(276) was isolated as a second distillate, which was collected at 132-136/0.1 mm(Hg) pressure as
a colourless oil (14.9 g, 55%) (Found: C, 53.0; H, 6.9; N, 5.39; 
M, 273; C₁₉H₁₉N₂O₆ requires: C, 52.74; H, 6.9; N, 5.13%, M, 273).

Route c(i)- Using methyl iodide:— Anhydrous potassium carbonate 
(11 g) was added to a solution of the ethoxycarbamate (205) (10.4 g) 
in acetone (30 ml) with stirring. Methyl iodide (7 g) was added
dropwise to the resulting green coloured solution and this solution 
was heated at reflux for 1 h. A precipitate of potassium iodide 
was removed and the mother liquors were diluted with ether (50 ml).
A second precipitate of potassium iodide was removed and the solution 
was evaporated to give an oily solid which failed to react with 
phenylethylamine. Vacuum distillation of this oily solid gave the 
required product in very low yield (5%) at 130/0.1 mm (Hg) pressure,
identical to the material produced by routes a) and b).

Route c(ii) Using diazomethane:— A suspension of the ethoxy-
carbamate (205) (10 g) in dry ether (50 ml) was magnetically stirred.
A solution of diazomethane in ether was slowly added over a 
period of 2 h until gaseous evolution has ceased and a permanent 
green colour remained. The excess diazomethane was destroyed by
the addition dropwise of acetic acid until visible reaction
ceased. The solution was evaporated to an oil. (Found: M, 287;
Calc. for C₈H₁₉N₂O₆; M, 273; Calc. for C₂H₉N₂O₆; M, 287)m/e 273(M - CH₄).

Route c(iii) Using dimethylsulphate:— A mixture of the 
ethoxycarbamate (205) (3 g) and dimethylsulphate (10 ml) was 
heated at 100 for 2 h. The resulting solution was evaporated and 
the remaining solid on recrystallisation from ethyl acetate gave 
crystals (identical m.p., mixed m.p., t.l.c.) to the original 
ethoxycarbamate (205).
Route c(iv) Using dimethyl sulphate and sodium ethoxide:—A solution of the ethoxycarbamate (205) (5.2 g) in alcohol (30 ml) was cooled to 0° using an ice/salt bath. Ethanolic sodium ethoxide (0.46 g sodium in 20 ml) was added and to the resulting green coloured solution was added dimethyl sulphate (2.6 g, 1.9 ml) dropwise over a period of 20 min. The final solution was maintained at 0° for several hours and then diluted with ether. The near quantitative precipitate of sodium methyl sulphate was removed and the solution was evaporated to an oil, a sample of which reacted vigorously with phenylethylamine. Ethyl N-(\(\alpha\)-ethoxycarbonyl-\(\beta\)-ethoxy-N-methylacryloyl)carbamate (276) was recovered by vacuum distillation of the oil, the vast majority of which distilled over at 132-136°/0.1 mm(Hg) pressure as a colourless oil, and was found to be identical (u.v., t.l.c., reaction products) with the material prepared by routes a) and b).

Route c(v) Using dimethyl sulphate, sodium ethoxide and silver nitrate:—To a solution of the ethoxycarbamate (205) (2.6 g) in alcohol (20 ml) was added an ethanolic solution of sodium ethoxide (0.23 g sodium in 10 ml). This solution was stirred and to it was added a solution of silver nitrate (1.9 g) in alcohol (30 ml). The flask was covered with black paper, even so the solution slowly turned black, giving a small amount of the silver salt as a pale grey precipitate which on standing gave a dark coloured gel which proved impossible to purify.

5-Ethoxycarbonyl-1-phenylethyl-3-methyluracil (283)

a) From the ethoxycarbamate (276) and phenylethylamine:—Phenylethylamine (1.4 g, 11 mmol) was added to a solution of the ethoxycarbamate (276) (2.73 g, 10 mmol) in ether (20 ml) at 0° and the
mixture was stirred. As the amine dissolved a vigorous exothermic reaction occurred, which resulted, on cooling, in the precipitation of the acyclic derivative (282) as a white powder, m.p. 94°, which was collected, washed with ether and heated to 100° for several minutes. Ethanol was evolved and the acyclic derivative cyclised to the 3-methyluracil ester (283) which gave needles from ethyl acetate, m.p. 110° (0.85 g). (Found: C, 63.2; H, 6.14; N, 9.03; M, 302; C₁₆H₁₅N₄O₂ requires: C, 63.58; H, 5.96; N, 9.27%; M, 302).

b) By direct methylation of the 5-ethoxycarbonyl-1-phenyl-ethyluracil (220): To a stirred suspension of 5-ethoxycarbonyl-1-phenylethyluracil (220) (2 g) in ether (15 ml) and dimethylformamide (15 ml) was added a solution of diazomethane in ether. As the yellow colour of the solution faded, so more diazomethane was added. The suspension slowly dissolved over 4 h, and the solution was set aside overnight. Excess diazomethane was removed by the addition of a small quantity of acetic acid and the solution was evaporated to an oil, which on trituration in water gave an amorphous solid (1.9 g).

Recrystallisation of this solid from ethyl acetate gave 5-ethoxycarbonyl-1-phenylethyl-3-methyluracil (283) as fine needles (0.9 g) identical (mixed m.p., m.p., i.r., u.v.) to the sample prepared from the ethoxycarbamate (276) and phenylethylamine.

5-Carboxy-1-phenylethyl-3-methyluracil (284)

To a solution of ethyl N-(α-ethoxycarbonyl-ν-ethoxy-N-methyl-acryloyl)carbamate (276) (2.73 g, 10 mmol) in ethanol (15 ml) was added phenylethylamine (1.4 g, 11 mmol) and the solution was warmed on a water bath for a few min. On dilution with water (20 ml), the solution precipitated an oil which was collected
and dissolved in aqueous sodium hydroxide solution (0.9 g, in 10 ml). The solution was warmed on a water bath for 2-3 min and then neutralised with 2M hydrochloric acid. 5-Carboxy-1-phenylethyl-3-methyluracil(284) was obtained as a precipitate (2.1 g) which gave needles from water, m.p. 248° and effervesced with aqueous sodium bicarbonate solution. (Found: M, 274; Calc. for C_{10}H_{14}N_{2}O_{4} M, 274); m/e 230(M - CO).  

5-Ethoxycarbonyl-3-methyluracil(287)  

Dry ammonia gas was bubbled into a solution of the ethoxy-N-methylcarbamate (276) (1.3 g) in ethanol (20 ml) until a saturated solution was obtained. This solution after standing at room temperature for 10 min was evaporated to give an oil, rich in the acyclic derivative (285), which was divided into two portions. One portion was dissolved in a solution of sodium ethoxide (0.23 g sodium) in ethanol (10 ml) and this solution was warmed on a steam bath for 15 min, diluted with water (10 ml) and adjusted to pH 6 with M sulphuric acid. The precipitate (0.3 g) of 5-ethoxycarbonyl-3-methyluracil (287) was collected and gave plates from ethyl acetate, m.p. 170°. (Found: M, 198; Calc. for C_{10}H_{10}N_{2}O_{4}; M, 198) m/e, 153(M - EtO); 126(M - CO_{2}Et).  

5-Carboxy-3-methyluracil(286)  

The second portion of the oil (285) was mixed with an aqueous solution of M sodium hydroxide (10 ml) and heated on a steam bath until a solution was formed (about 10 min). This solution was cooled and gave a precipitate of the sodium salt of the required acid. The precipitate was redissolved by warming and the solution was neutralised with M sulphuric acid. The resulting precipitate of 5-carboxy-3-methyluracil(286) was redissolved by
boiling and this solution gave the acid as a chunky crystalline
material on cooling, m.p. 220°C (decomp) (Found: M⁺, 170; Calc. for
C₆H₆N₂O₄; M, 170) m/e 126(M - CO₁+.

5-Carboxy-1,3-dimethyluracil (289)

To a solution of the ethoxy N-methylcarbamate (276) (1.4 g)
in ether (20 ml) was added a 33% ethanolic solution of methylamine
(0.9 g). This solution was warmed on a steam bath for a few min
and evaporated to an oil, which failed to solidify when tritur-
ated in ether except when cooled to -77°C. The ether was removed
and the oil, which would not dissolve in water, slowly dissolved
in warm aqueous 2M sodium hydroxide solution. The resulting
yellow coloured solution was adjusted to pH 6.2 with M sulphuric
acid and gave a precipitate which redissolved on heating. 5-Carboxy-
1,3-dimethyluracil (289) was obtained as crystals from this solution
on cooling, m.p. 212°C (Found: M⁺, 184; Calc. for C₇H₆N₂O₄; M, 184),
m/e 140(M - CO₂+.

Decarboxylation of the N-methyluracil acids (284), (286), and (289)

a) By heating in air: A small quantity of each acid was
heated, using an oil bath, to 200°C and kept at that temperature
for 20 min. No visible change was seen, however, recrystallisation
from water in each case gave a material, different to the starting
material (m.p., t.l.c.) and identical to the products of b).

b) By heating in N-ethylmorpholine: In each case a small
quantity of the N-methyl acid (284), (286) or (289) was suspended
in N-ethylmorpholine and the suspension was heated to 130-135°C
until gaseous evolution ceased (about 10-15 min). The solid was
recovered and recrystallised from water to give 1-phenylethyl-
3-methyluracil (290a) m.p. 253°C (Found: M⁺, 230; Calc. for C₇H₆N₂O₄
M, 230); 3-methyluracil(290b), m.p.234°(Found: M, 126; Calc. for C₅H₄N₂O₂; M, 126) and 1,3-dimethyluracil(290c), m.p.217°(Found: M, 140; Calc. for C₆H₆N₂O₂; M, 140) respectively. These products were found to be identical (m.p., mixed m.p., m.s.) with the products of the above decompositions in a).

α-Cyano-β-anilino-N-formylacrylamide(293a)

Aniline (0.45 g) was added to a solution of α-cyano-β-ethoxy-N-formylacrylamide(291) (0.89 g) in alcohol (15 ml). The mixture was stirred and warmed, and a solution was produced which gave a yellow precipitate, m.p.162°, after a few minutes. Recrystallisation from ethyl acetate gave α-cyano-β-anilino-N-formylacrylamide(293a) as white needles, m.p. 168°(Found: C, 61.58; H, 4.3; N, 19.31; M, 215; Calc. for C₁₁H₉N₃O₂: C, 61.4; H, 4.19; N, 19.53% M, 215)

(i.r. :CN absorption peak at ν max 2245 cm⁻¹)

Attempted cyclisation of α-cyano-β-anilino-N-formylacrylamide(293a)

a) Heating in air:— A small sample of (293a) was heated until it melted at 162° and maintained at this temperature for 4-5 min. On cooling the product was found to be identical (i.r.) to the starting material.

b) Using ammonia solution:— To a suspension of the acrylamide in warm water was added a few drops of ammonium hydroxide solution. The yellow material lost its colour to yield a white powder, m.p. 220°(Found: C, 63.85; H, 5.24; N, 22.94; M, 215; Calc. for C₁₁H₇N₃O C, 67.0; H, 3.55; N, 21.32% M, 197).

c) Heat with barium oxide:— A small sample of the acrylamide (1 g) was mixed with solid barium oxide (2.5 g) and heated at 80-90° for 3 h. The i.r. spectrum of the benzene extract of this mixture was found to be identical to that of the starting material.
d) Heat with calcium oxide: An intimate mixture of the acrylamide (293a) (1 g) and calcium oxide (1.5 g) was heated at 220-230°C for 18 h under a nitrogen atmosphere. Some decomposition occurred but the benzene extract of the final mixture possessed an i.r. spectrum identical to that of the starting material.

e) Pyrophosphoryl chloride: Pyrophosphoryl chloride was prepared by heating a mixture of phosphorous pentoxide (250 g) and phosphorous pentachloride (500 g) at 105°C for 8 h, filtering, and distilling the filtrate at 96-98°C (under water pump vacuum) (yield 80 g). An intimate mixture of the pyrophosphoryl chloride (2.7 ml) and the acrylamide (0.43 g) was mixed with dry carbon tetrachloride (10 ml) and the mixture was warmed on a steam bath for 20 min. The carbon tetrachloride was evaporated off and dilute sodium hydroxide solution was added to produce an alkaline solution, which was neutralised with dilute sulphuric acid. On evaporation the solution gave an oil which despite cooling and scratching, failed to crystallise.

\(\alpha\)-Cyano-\(\beta\)-methylamino-N-formylacrylamide (293b)

To a warm solution of \(\alpha\)-cyano-\(\beta\)-ethoxy-N-formylacrylamide (291) (1 g) in ethyl acetate (15 ml) was added methylamine (2 ml) until the smell of the base persisted. The solution was cooled and after 6 h gave a white powder, m.p. 178°C, which gave \(\alpha\)-cyano-\(\beta\)-methylamino-N-formylacrylamide (293b) (0.78 g) as plates, m.p. 184°C from ethyl acetate. (Found: \(M^+\), 153; Calc. for \(C_{6}H_{11}N_{3}O\): \(M^+\), 153) (i.r.: \(-CN\) absorption peak at \(\nu_{\text{max}}\) 2240 cm\(^{-1}\)).

Attempted cyclisation of (293b)

Methods analogous to those used in an attempt to cyclise (293a) were used, none however were successful.
α-Cyano-β-cyclohexylamino-N-formylacrylamide(293c)

To a warm solution of α-cyano-β-ethoxy-N-formylacrylamide (291) (1 g) in ethyl acetate (15 ml) was added cyclohexylamine (0.6 g). The solution darkened from pale yellow to deep green. No crystalline deposit was produced on standing overnight, the solution was evaporated to low volume, cooled, and scratched. When this failed to produce a crystalline material, the solution was rapidly cooled in a freezing box of a refrigerator. A white solid was obtained which redissolved as the solution warmed to room temperature. This solid was collected (m.p. 170-180°) and found to be rather sticky. Attempted recrystallisations from nitromethane, ethyl acetate and methanol failed to give crystals. (Found: M, 220 (v. small), 208, 194 (v. large); Calc. for C_{14}H_{15}N_{3}O_{2}: M, 221).

5-Cyano-2-methyl-2-deoxyuracil(296a)

To a solution of α-cyano-β-ethoxy-N-acetylacrylamide (292) (1 g) in alcohol (10 ml) was added a 1% solution of ammonium hydroxide (15 ml). A red coloured solution was formed, which failed, on cooling, to yield a crystalline material. The solution was evaporated to dryness leaving a red oily solid which was heated under reflux with ethyl acetate (15 ml) for 20 min, leaving a green coloured solution and a red coloured solid. Evaporation of the green solution gave a small quantity of crystalline material, m.p. 180-185° (Found: M, 315; Calc. for (296a) M, 135) The red solid when recrystallised from water gave 5-cyano-2-methyl-2-deoxyuracil (296a) (0.5 g) as an almost colourless crystalline material, m.p. 243° (Found: C, 53.1; H, 3.81; N, 31.15; M, 135; Calc. for C_{6}H_{5}N_{3}O: C, 53.3; H, 3.70; N, 31.1%, M, 135) (i.r. strong -CN absorption at v max 2235 cm).
α-Cyano-β-anilino-N-acetylacrylamide (295b)

To a solution of α-cyano-β-ethoxy-N-acetylacrylamide (292) (1 g) in alcohol (10 ml) was added aniline (1 g). After warming for a few seconds crystals, m.p. 202-204° (decomp), appeared which were collected and on recrystallised from methanol gave α-cyano-β-anilino-N-acetylacrylamide (295b) (0.45 g) as needles, m.p. 206° (decomp). (Found: C, 63.1; H, 4.72; N, 18.35; M, 229; \( \text{C}_{11}\text{H}_7\text{N}_3\text{O} \) requires : C, 62.88; H, 4.80; N, 18.34% M, 229).

Attempted cyclisation of (295b)

a) Ammonium hydroxide: - To a suspension of (295b) (1 g) in hot water (10 ml) was added dropwise, a dilute solution of ammonium hydroxide. A colour change was noted in the suspended material (strong yellow colouration faded). The solid was recovered and gave needles, m.p. > 280° from methanol. (Found: C, 68.13; H, 5.16; N, 19.12; \( \text{C}_{11}\text{H}_7\text{N}_3\text{O} \) requires C, 68.24; H, 4.27; N, 19.91%)

b) Heat: - A small sample of the acetylacrylamide (295b) was heated to 230° and maintained at this temperature for 4-5 min. The solid melted and rapidly darkened. The resulting black tar was treated with nitromethane, methanol and dilute sodium hydroxide solution followed by dilute sulphuric acid, however, it proved impossible to recrystallise.

Ethyl N-(α-cyano-β-anilinoacryloyl)dithiocarbamate (297a)

To a solution of 5-cyano-2-ethylthio-4-oxo-1,3-thiazine (72) (1 g) in warm benzene (10 ml), was added aniline (0.5 g) and the solution was cooled. Crystals of ethyl N-(α-cyano-β-anilinoacryloyl)dithiocarbamate (297a) (1.1 g) were deposited, which gave yellow laths, m.p. 154° (resolidified then 270°), from ethanol. (Found: C, 53.72; H, 4.46; N, 14.26; M, 291. \( \text{C}_{14}\text{H}_{16}\text{N}_3\text{OS} \) requires
C, 53.5; H, 4.47; N, 14.43 %; M, 291)(i.r. showed very strong absorption corresponding to $-\text{CN}$ at $\nu_{\text{max}}$ 2200 cm$^{-1}$).

5-Carboxamido-1-phenyl-2-thiouracil(299)

The dithiocarbamate(297a) (1 g) was dissolved in 0.5 M sodium hydroxide solution (10 ml) and the resulting solution was maintained at 80-100 °C for 10 min. The solution was neutralised with M sulphuric acid and a precipitate of 5-carboxamido-1-phenyl-2-thiouracil(299) (0.6 g) was produced, which was collected and recrystallised from acetic acid to give 5-carboxamido-1-phenyl-2-thiouracil as plates, m.p. 284 °C. (Found: C, 53.26; H, 3.60; N, 16.83; M, 247; Calc. for C$_{14}$H$_9$N$_3$O$_2$S; C, 53.44; H, 3.64; N, 17.0 %; M, 247) (u.v. absorption peaks at $\lambda_{\text{max}}$ (pH 7) 311 nm, $\lambda_{\text{max}}$ (pH 10) 323 nm).

5-Cyano-1-phenyl-2-thiouracil(298a)

A sample of the dithiocarbamate(297a) (1 g) was dissolved in 0.25 M sodium hydroxide solution (10 ml) which was immediately neutralised with M sulphuric acid. The precipitate formed was collected and gave 5-cyano-1-phenyl-2-thiouracil(298a) as plates, m.p. 280 °C, from ethyl acetate. (Found: C, 57.70; H, 3.2; N, 18.28; M, 229; C$_6$H$_7$N$_3$OS requires C, 57.64; H, 3.06; N, 18.34 %; M, 229). (I.r. strong $-\text{CN}$ absorption at $\nu_{\text{max}}$ 2205 cm$^{-1}$; u.v. $\lambda_{\text{max}}$ (pH 7) 312 nm, $\lambda_{\text{max}}$ (pH 10) 294 nm).

5-Carboxamido-1-phenyl-2-deoxyuracil(300)

To a solution of 5-carboxamido-1-phenyl-2-thiouracil(299) (0.5 g) in ethanol (10 ml) was added Raney nickel (4 g wet weight). The mixture was heated under reflux for 20 min. A white precipitate separated and the solution was cooled and filtered. The solid was extracted with ethyl acetate (15 ml) from which 5-carboxamido-1-phenyl-2-deoxyuracil(300) was obtained as pale green laths (0.3 g), m.p.
265. (Found: C, 61.41; H, 3.98; N, 19.71; M, 215; C₁₀H₉N₂O₁₂ requires: C, 61.4; H, 4.19; N, 19.53% M, 215) (u. v. λ max (pH 7 and 10), 314 nm).

5-Cyano-1-phenyl-2-deoxyuracil (29αa)

To a solution of 5-cyano-1-phenyl-2-thiouracil (29αa) (1 g) in alcohol (50 ml) was added Raney nickel (type 101)(4 g). The mixture was heated under reflux for 90 min, during which time the progress of the reaction was followed by u. v. spectroscopy. The nickel was removed, and on evaporation and cooling, the solution gave 5-cyano-1-phenyl-2-deoxyuracil as crystals, m. p. 280°. (Found: C, 67.05; H, 3.51; N, 21.25; M, 197; C₁₀H₉N₂O requires: C, 67.0; H, 3.55; N, 21.32% M, 197) (u. v., λ max (pH 7), 320 nm, λ max (pH 10) 323 nm; i. r. strong -CN absorption v max 2210 cm⁻¹).

Ethyl N-(α-cyano-β-cyclohexylaminoacryloyl)dithiocarbamate (297b)

To a warm solution of 5-cyano-2-ethylthio-4-oxo-1,3-thiazine (72) (0.5 g) in benzene (5 ml) was added cyclohexylamine (0.3 g). A yellow oil remained after the solvent was removed, which proved impossible to crystallise. The experiment was repeated using methanol (10 ml) as solvent, which was set aside for 2 days. Ethyl N-(α-cyano-β-cyclohexylaminoacryloyl)dithiocarbamate (297b) was obtained as chunky crystals (0.3 g), m. p. 280° (resolidified then 264°). (Found: M, 297; Calc. for C₁₀H₉N₂OS: M, 297) (i. r. strong -CN absorption at v max 2210 cm⁻¹).

5-Carboxamido-1-cyclohexyl-2-thiouracil (301)

A solution of the above dithiocarbamate (297b) (0.1 g) in 0.5 M sodium hydroxide solution (5 ml) was warmed on a steam bath for 5 min, and then neutralised with M sulphuric acid. A precipitate was immediately produced which was collected and recrystall-
ised from ethyl acetate, from which 5-carboxamido-1-cyclohexyl-2-thio-
uracil separated as plates, m.p. 270° (Found: M⁺, 253; Calc. for
C₁₉H₁₅N₃O₅S : M, 253) (u.v. λ max(pH7) 323 nm, λ max(pH10) 305 nm).

5-Cyano-3-methyl-1-p-chlorobenzyluracil(311)

Solutions of ethyl N-(α-cyano-β-ethoxy-N-methylacryloyl)carb-
amate(67) (2.1 g) in alcohol (5 ml) and p-chlorobenzylamine(304)
(1.41 g) in alcohol (5 ml) were mixed. An exothermic reaction
occurred, and the mixed solution was heated under reflux for 15 min.

On cooling 5-cyano-3-methyl-1-p-chlorobenzyluracil(311) was
deposited as a chunky white crystalline material (2.1 g), m.p.
181° (Found: C, 56.53; H, 3.70; N, 15.12; M, 275; C₁₃H₁₀N₃O₂Cl requires:
C, 56.6; H, 3.63; N, 15.4%; M, 275) (u.v. λ max(pH7) 279, 224 nm,
and λ max(pH10) 276, 222 nm; i.r. -CN absorption ν max 2235 cm⁻¹).

5-Cyano-3-methyl-1-o-chlorobenzyluracil(312)

To a solution of ethyl N-(α-cyano-β-ethoxy-N-methylacryloyl)-
carbamate(67) (2.7 g) in alcohol (5 ml) was added o-chlorobenzyl-
amine(305) (2 g). The solution warmed rapidly and was heated
under reflux for 10 min. During this time a precipitate was formed,
which thickened on cooling, and which gave 5-cyano-3-methyl-1-o-
chlorobenzyluracil as plates (2.7 g), m.p. 160° from nitromethane.
(Found: M⁺, 275; Calc. for C₁₃H₁₀N₃O₂Cl : M, 275), m/e 240(M - Cl);
(i.r. -CN absorption ν max 2230 cm⁻¹; u.v. λ max(pH7) 278, 224 nm;
λ max(pH10), 274, 223 nm).

5-Cyano-3-methyl-1-(4'-imidazole ethyl)uracil(313)

To a suspension of histamine hydrochloride(306) (2.3 g) in
methanol (10 ml) was added a methanolic solution of sodium (30 ml)
(2 g per 100 ml). The mixture was warmed and stirred. The suspension
dissolved and a precipitate of sodium chloride was produced, which
was removed. The filtrate was added to a solution of ethyl N-(α-cyano-β-ethoxy-N-methylacryloyl)carbamate (2.8 g) in methanol (5 ml) and the mixed solution was warmed on a steam bath for 10 min. Evaporation of the solvent and dilution with ether (10 ml) precipitated an oil which crystallised over 2 days and gave 5-cyano-3-methyl-(4'-imidazoleethyl)uracil as needles (2.1 g), m.p. 196°, from nitromethane. (Found: M⁺, 245; Calc. for C₁₀H₁₉N₃O, M⁺, 245) (i.r. -CN absorption at ν max 2240 cm⁻¹; u.v. λ max (pH7) 285, 228 nm; λ max (pH10) 283 and 228 nm).

5-Cyano-3-methyl-1-(4'-hydroxyphenylethyl)uracil(314)

To a suspension of the tyramine hydrochloride (2.7 g) in alcohol (10 ml) was added alcoholic sodium ethoxide solution (18 ml) (2 g per 100 ml). The suspension dissolved and the precipitated sodium chloride was removed. The filtrate was mixed with a solution of ethyl N-(α-cyano-β-ethoxy-N-methylacryloyl)carbamate (3.5 g) in alcohol (7 ml) and the mixed solution was heated on a steam bath for 5 min. During this time a thick crystalline precipitate was obtained which gave 5-cyano-3-methyl-1-(4'-hydroxyphenylethyl)uracil (314) as fine needles (2.1 g), m.p. 198°, from nitromethane. (Found: C, 62.1; H, 4.85; N, 15.56; M⁺, 271; C₁₀H₁₉N₃O₃ requires: C, 62.0; H, 4.80; N, 15.5%) (i.r. -CN absorption at ν max 2240 cm⁻¹; u.v. λ max (pH7), 285, 217 nm; λ max (pH10) 286, 216 nm).

5-Cyano-3-methyl-1-(3',4'-dihydroxyphenylethyl)uracil(315)

To a suspension of 3-hydroxytyramine hydrochloride (3.0 g) in alcohol (10 ml) was added an alcoholic solution of sodium ethoxide (18 ml) (2 g per 100 ml). A bright purple colour was produced in the solution which darkened to brown. The suspension
dissolved and the sodium chloride precipitate was removed. The
dark brown coloured filtrate was mixed with a solution of ethyl-
N-(α-cyano-β-ethoxy-N-methylacryloyl)carbamate (67) (3.5 g) in
alcohol (7 ml). An immediate precipitate began to form, which
thickened as the mixture was warmed, on a steam bath, for 5 min.
The thick precipitate was collected and gave 5-cyano-3-methyl-
1-(3',4'-dihydroxyphenylethyl)uracil (315) as needles (3.2 g), m.p.
204° from water. (Found: M⁺, 287. Calc. for C₁₃H₁₃N₃O₂: M⁺, 287) (i.r.
-CN absorption at ν max 2243 cm⁻¹; u.v. λ max (pH7) 288, 231 nm; λ max
(pH10), 284, 228 nm).

5-Cyano-3-methyl-1-(indole-3'-ethyl)uracil (316)

To a suspension of tryptamine hydrochloride (309) (3 g), in
alcohol (10 ml) was added an alcoholic solution of sodium ethoxide
(18 ml) (2 g per 100 ml). The suspension dissolved and the precip-
itate of sodium chloride was removed leaving a pale green filtrate,
which was mixed with a solution of ethyl N-(α-cyano-β-ethoxy-N-
methylacryloyl)carbamate (67) (3.5 g) in alcohol (7 ml). An immediate
white precipitate was produced which thickened as the mixture
was heated on a steam bath for 5 min. This crystalline precipitate
gave 5-cyano-3-methyl-1-(indole-3'-ethyl)uracil (316) as plates
(3.1 g), m.p. 236° from water. (Found: M⁺, 294; Calc. for C₁₉H₁₅N₄O₂;
M, 294) (i.r. -CN absorption at ν max 2238 cm⁻¹; u.v. λ max (pH7),
279, 223 nm; λ max (pH10) 276, 215 nm).
Experimental details relating to the synthesis, or attempted
synthesis, of pyrazolo[3,4-d]pyrimidines contained in chapters
nine to fourteen of the discussion.
Ethyl N-(α-cyano-β-ethoxyacryloyl)carbamate (124)

Ethyl N-cyanoacetylcarbamate (Conrad and Schulze, loc. cit) (3.12 g), triethyl orthoformate (2.96 g), and acetic anhydride (5 ml) were boiled under reflux for 1 h, the crystalline product (124) separated on cooling, was washed with light petroleum, and crystallised from benzene as needles (3.2 g), m. p. 120°.

Ethyl N-(α-cyano-β-hydrazinoacryloyl)carbamate (320)

Hydrazine hydrate (0.28 g) in ethanol (1 ml) was added to a warm solution of ethyl N-(α-cyano-β-ethoxyacryloyl)carbamate (124) (1 g) in ethanol (10 ml). The immediate pale yellow precipitate was collected and washed with ethanol and ether to give the hydrazinocarbamate (320) (0.95 g), which decomposed from 125° when heated quickly but rapidly resolidified and then had m. p. > 300°. (Found: C, 42.35; H, 5.25; N, 28.1; C_{10}H_{16}N_9 requires C, 42.4; H, 5.05; N, 28.28 %) (i. r. ν max 2240 cm⁻¹ (CN); u. v. λ max 282, 216 nm).

Pyrazolo[3,4-d]pyrimidine-4,6-dione (94a)

a) From the hydrazino derivative (320) :- The foregoing hydrazino derivative (0.5 g) was heated at 140° (bath) for 10 min. There was little obvious change in the material and a pale yellow solid remained. This was crystallised from water to give the pyrazolopyrimidine (94a) (0.35 g) as needles, m. p. > 300°. (Found: C, 39.35; H, 2.85; N, 36.6; M⁺ 152; C_{5}H_{7}N_{2}O_{2} requires C, 39.47; H, 2.63; N, 36.84 %; M, 152) (u. v. λ max 268, 243 nm). This material was found to be identical (mixed m. p., i. r., u. v.) with an authentic sample prepared by the Robins route below.

b) By the Robins route :- To a solution of sodium (2 g) in ethanol (75 ml) was added formamidine hydrochloride (10 g) and cyanoacetamide (8.4 g), and the mixture was stirred at 15° for 3 h.
The resulting solution of the aminomethylene derivative (325) was raised to 70° and an aqueous solution of hydrazine hydrate (6 g in 50 ml) was added. The temperature was maintained at 70° for a further 15 min. The solution was then cooled by the addition of ice (20 g) and acidified with concentrated sulphuric acid (6 ml). A precipitate of 5-aminopyrazole-4-carboxamide (92a) (16.3 g) was obtained which was collected, recrystallised from water and washed with water and acetone, m.p. 230-233°.

A mixture of urea (4.6 g) and the foregoing pyrazole (2.8 g) was heated at 160° for 5 min. It melted, effervesced and resolidified. The amorphous material was dissolved in 0.5 M sodium hydroxide solution and the resulting solution was filtered, and neutralised with acetic acid. The pyrazolopyrimidine (94a) was precipitated as a powder identical with the material prepared above.

5-Aminopyrazole-4-(N-ethoxycarbonyl)carboxamide (321)

Recrystallisation of the foregoing hydrazinoacyclic compound (320) from ethyl acetate gave the pyrazole (321) as laths. This product gave a coloured derivative with the Bratton Marshall test with λmax 491 nm. A sample of the aminopyrazole (321) was heated quickly to 130° and maintained at this temperature for 10 min, it melted, effervesced, and rapidly resolidified. During normal m.p. determination the 130° figure may go unnoticed. (U.v. λmax 261 nm at pH 10).

1-Methylpyrazolo[3,4-d]pyrimidine-4,6-dione (94b)

a) From the acryloylcarbamate reagent (124) :- Solutions of methyl hydrazine (1 g) in nitromethane (5 ml) and ethyl N-(α-cyano-β-ethoxyacryloyl)carbamate (124) (4.35 g) in nitromethane (25 ml) were mixed at 5° and allowed to warm to room temperature.
overnight. The pale yellow crystalline precipitate formed, gave 5-amino-1-methylpyrazole-4-(N-ethoxycarbonyl)carboxamide (323) (0.3 g) as laths, m.p. 300°C (from water). (Found: C, 45.2; H, 5.81; N, 26.3; C₇H₅N₃O₂ requires: C, 45.28; H, 5.66; N, 26.42%). Rf 0.7, no CN absorption. The product gave a coloured product in the Bratton-Marshall test with λ_max 483 nm.

A sample of the aminopyrazole (323) was heated quickly to 160°C and maintained at this temperature for 10 min; it melted, effervesced, and rapidly resolidified. During a normal m.p. determination the figure of 160°C may go unnoticed. The residue was purified by dissolution in dilute aqueous potassium hydroxide and precipitation with acetic acid to give the pyrazolopyrimidine (94b) in almost quantitative yield, as needles, m.p. 310°C (Found: C, 43.5; H, 3.51; N, 33.9, M, 166. C₆H₅N₄ requires: C, 43.37; H, 3.61; N, 33.73% M, 166). Rf 0.6; λ_max (alkaline solution) 247 and 269 nm, λ_max (pH 7) 256 nm, no CN absorption. This product was found to be identical with that prepared by the Robins route detailed below. Ethyl N-(α-cyano-β-methylhydrazinoacryloyl)carbamate (322) was found to be present in the original solution giving rapidly weakening absorption peaks at 2240 cm⁻¹ (CN) and λ_max 278 and 212 nm as the molecule rearranged to the pyrazole (323).

b) By the Robins route: To a solution of methyl hydrazine (70 g) in ethanol (200 ml) was carefully added ethoxymethylene malononitrile (121 g). The rate of addition being such that the solution was maintained at the reflux. A white precipitate gradually appeared. The reaction mixture was heated on a steam bath for 30 min and then placed in a refrigerator overnight. 5-Amino-4-cyano-1-methylpyrazole (94b) was collected and washed with a little cold
ethanol, m.p. 221-223° (109 g).

To 100 ml of concentrated sulphuric acid, cooled in an ice bath, was gradually added, with stirring, the foregoing pyrazole (91b) (40 g). The reaction temperature was maintained at 15-20° and the addition was completed after 2 h. The final solution was stirred at room temperature for 30 min and then poured with stirring into crushed ice (500 g), then adjusted to pH 8 with concentrated ammonium hydroxide. The temperature being maintained below 50° by the addition of more crushed ice. 5-Amino-1-methylpyrazole-4-carboxamide (92b) separated overnight on cooling, as colourless crystals (30 g), m.p. 232-235°, which gave crystals from water, m.p. 237-239°.

5-Amino-1-methylpyrazole-4-carboxamide (92b) (50 g) and urea (100 g) were fused together at 180-200° for 1 h. The mixture melted, thickened and finally, with continuous agitation, resolidified. The cooled solid mass was dissolved in hot dilute potassium hydroxide solution (1000 ml) and then the solution was carbon treated, filtered and acidified with glacial ethanoic acid. The pyrazolopyrimidine (94b) separated as a white precipitate (55 g) (93%), m.p. >300°, and was found to be identical (u.v., i.r., mixed m.p.) with the pyrazolopyrimidine (94b).

1-Phenylpyrazolo[3,4-d]pyrimidine-4,6-dione (94c)

a) From the acryloylcarbamate reagent (124) :- A solution of the acryloylcarbamate (124) (1 g) in ethanol (5 ml) containing phenylhydrazine (0.5 g) was set aside. A red precipitate was obtained which was collected and decolourised by washing with ether. Ethyl N-(α-cyano-β-phenylhydrazinoacryloyl)carbamate (125) (0.65 g) was obtained as plates, m.p. 128-129° (decomp). (Found: C, 56.9;
H, 5.15; N, 20.65; C_{11}H_{15}N_{12}O_{3} requires: C, 56.93; H, 5.11; N, 20.44%; υ max 2200 cm⁻¹(CN).

A portion of the compound was dissolved in hot ethanol. The cooled solution deposited crystals of 5-amino-1-phenylpyrazole-4-(N-ethoxycarbonyl)carboxamide(130), which gave prisms from ethanol, i.r. analysis indicated no CN absorption. The product gave a coloured product with the Bratton Marshall test with λ max 494 nm. The compound had no definite m.p. but when a portion was immersed in a tube in a bath at 180° it rapidly liquefied, then resolidified to give the pyrazolo[3,4-d]pyrimidine(94c), which gave needles, m.p. 340° (from ethanol and water). (Found: C, 58.1; H, 3.51; N, 24.3; C_{11}H_{15}N_{12}O_{3} requires: C, 57.89; H, 3.51; N, 24.56%); λ max 236 nm.

The same compound was obtained by heating the phenylhydrazino carbamate(125), at 160° for 5 min, it melted with effervescence and resolidified. The i.r. spectrum showed no CN absorption. This compound was found to be identical (m.p. mixed m.p., i.r., u.v.) with a sample prepared by the method of Cheng and Robins.

b) By the Robins route:— To a solution of phenylhydrazine (88 g) in absolute ethanol (360 ml) was added slowly, and with shaking, ethoxymethylene malononitrile (100 g). After about half of the addition was complete the solution was carefully heated to boiling. The remaining malononitrile was added so as to maintain gentle boiling of the solution and the final solution was heated at reflux for a further 30 min. and set aside overnight in the refrigerator. The product was filtered (120 g) and washed with a little ether. The product gave white crystals, m.p. 140°, of 5-amino-4-cyano-1-phenylpyrazole(91c), from water.

To 400 ml of concentrated sulphuric acid cooled in an ice bath
was added, with stirring, the foregoing cyanopyrazole (91c) (88 g). During the addition, which required 3 h, the reaction temperature was maintained at 10-15°. The final mixture was stirred at room temperature until solution was complete. The dark sulphuric acid solution was then poured onto crushed ice, and the solution was neutralised with concentrated ammonium hydroxide. The solution, which was allowed to reach 65-70° during neutralisation, was cooled to room temperature and filtered to give 5-amino-1-phenylpyrazole-4-carboxamide (92c) as a yellow crystalline product (90g), m.p. 169-170°, which gave crystals, m.p. 172-173° from water.

An intimate mixture of the foregoing phenylpyrazole (92c) (50 g) and urea (100 g) was fused at about 200°, until the fused mass resolidified. The cooled melt was dissolved in dilute sodium hydroxide solution (1000 ml) and the solution was carbon treated, filtered, and acidified with acetic acid. The resulting white precipitate was collected, washed with water and dried at 130° (53 g, 94%), m.p. >300°. The required phenylpyrazolopyrimidine (94c) was obtained as long white needles, m.p. 320-321°, from 80% aqueous acetic acid and was found to be identical (i.r., u.v., mixed m.p.) to the pyrazolopyrimidine prepared above.

N-Cyanoacetylformamide (326)

A mixture of cyanoacetic acid (17 g) and acetic anhydride (15 ml) was maintained at 70° until a clear solution was obtained (10-15 min). Formamide (4.8 g) was added and the solution was kept at 70-75° for 3 h, then evaporated to a brown syrup, which soon crystallised. The product was triturated with a small amount of cold ethyl acetate, and the solid was filtered off and washed with cold ethyl acetate and ether. N-Cyanoacetylformamide (326)
(7 g) formed needles, m.p. 139-140° (from ethyl acetate). (Found: C, 42.8; H, 3.65; N, 24.85; \( C_4H_7N_2O_2 \) requires: C, 42.86; H, 3.57; N, 25.0%). A further quantity (1.1 g) of the formamide separated later from the mother liquors.

\( \alpha \)-Cyano-\( \beta \)-ethoxy-N-formylacrylamide (291)

a) A mixture of N-cyanoacetylformamide (326) (6 g), triethyl orthoformate (10 ml) and acetic anhydride (25 ml) was kept at 80° for 3 h, then evaporated to a syrup which rapidly crystallised. The solid formylacrylamide (291) (6 g) was triturated with cold ethyl acetate, filtered off, washed with a little cold ethyl acetate followed by ether or light petroleum (b.p. 40-60). (Found: C, 50.05; H, 4.7; N, 16.55, M, 168; \( C_7H_9N_2O_3 \) requires C, 50.0; H, 4.77; N, 16.66%; M, 168). Further crops of the acrylamide (1.2 g and 0.3 g) separated from the mother liquor, total yield 7.6 g (84.5%). The compound had \( \nu_{\text{max}} \) 2240 cm\(^{-1}\) (CN).

b) A mixture of cyanoacetic acid (34 g), acetic anhydride (80 ml) and formamide (20 ml) was heated on a water bath for 2 h at 100°. Acetic anhydride (90 ml) and triethyl orthoformate (66 ml) were added and the mixture was heated for a further 2 h, then evaporated to a small volume and cooled while crystals separated. These were collected and washed with a little cold ethyl acetate, to give the ethoxyacrylamide (291) (15 g), m.p. 132° identical (mixed m.p., i.r.) with the compound prepared in (a). Recrystallisation of (291) from ethyl acetate gave needles of the ethoxyacrylamide, however, recrystallisation from methanol gave \( \alpha \)-cyano-\( \beta \)-ethoxyacrylamide (327) by hydrolysis, identical (m.p., mixed m.p. u.v.) with a sample prepared from cyanoacetic acid and triethyl-orthoformate.
\( \alpha\)-Cyano-\( N\)-formyl-\( \beta\)-hydrazinoacrylamide(328)

The foregoing ethoxyacrylamide (1 g) in warm ethanol (10 ml) and hydrazine hydrate (0.3 ml) in ethanol (1 ml) were mixed to produce an immediate pale yellow precipitate of the hydrazinoacrylamide(328) (0.75 g), which was collected and washed with ethanol and ether. The product was pure (t. l. c.) although not readily recrystallised, and had m. p. \( \geq 320^\circ \) (Found: C, 39.1; H, 4.15; N, 36.2 \( \text{C}_5\text{H}_6\text{N}_4\text{O}_1 \) requires C, 38.96; H, 3.90; N, 36.36%), \( \lambda_{\max} 2240 \text{ cm}^{-1} \text{(CN)}. \)

Recrystallisation of the hydrazinoacrylamide(328) from nitromethane gave 5-aminopyrazole-4-\((N\)-formyl\)carboxamide(329) as plates isomeric with (328), but with no CN absorption. (329) with the Bratton Marshall reagents gave a highly coloured derivative (\( \lambda_{\max} 520 \text{ nm} \)).

Pyrazolo\([3,4-d]\)pyrimidine-4-one, allopurinol (93a)

a) The foregoing hydrazinoacrylamide(328) (1 g) was heated at 150\( ^\circ \) (bath) for 15 min. There was little or no visible change in the material. The residue was extracted with dilute aqueous sodium hydroxide solution and the extract was treated with decolourising charcoal and filtered. The filtrate was adjusted to pH 5 with hydrochloric acid to produce a crystalline precipitate. Allopurinol(93a) (0.75 g) was obtained as a crystalline powder by repetition of this process, m. p. \( \geq 320^\circ \) (Found: C, 44.05; H, 2.9; N, 41.05; \( \text{C}_5\text{H}_4\text{N}_4\text{O} \) requires: C, 44.1; H, 2.94; N, 41.2%). The compound had no CN absorption and was identical (i.r. and u.v., t. l. c) with an authentic specimen prepared below.

b) Cheng and Robins route: - A solution of 5-aminopyrazole-4-carboxamide(92a) (40 g) in formamide (100 ml) was heated to reflux for 2 h. An equal volume of water was added and the cooled solution
was set aside on a refrigerator overnight. The crude precipitate was collected, dissolved in hot dilute aqueous potassium hydroxide and neutralised with acetic acid. The pyrazolopyrimidine (93a) separated as a crystalline powder (36 g), m.p. >300°C, which gave crystals from water identical (mixed m.p., i.r., u.v.) with the specimen prepared in (a).

1-Phenylpyrazolo[3,4-d]pyrimidine-4-one (93c)

a) From the formylacrylamide reagent (291) :- Solutions of the foregoing formylacrylamide (291) (1 g) in ethanol (25 ml) and phenyl hydrazine (0.55 ml) in ethanol (1 ml) were mixed at 5°C and set aside overnight. A crystalline precipitate of α-cyano-N-formyl-β-phenyl-hydrazinoacrylamide (330) (1.5 g) formed as needles, m.p. 220°C (decomp). (Found: C, 57.15; H, 4.65; N, 24.2; C₁₅H₁₅N₄O₂ requires: C, 57.39; H, 4.35; N, 24.35%), νmax 2240 cm⁻¹ (CN). Crystallisation of the compound from nitromethane gave 5-amino-1-phenylpyrazole-4-(N-formyl)carboxamide (331) (1.3 g) as plates, m.p. 220°C (decomp). (Found: C, 57.2; H, 4.5; N, 24.35; C₁₅H₁₅N₄O₂ requires C, 57.39; H, 4.35; N, 24.35%) no CN absorption, Bratton Marshall test gave a coloured derivative (λmax 531 nm).

A sample of the aminopyrazole was maintained at 220-230°C (bath) for 10 min, there was rapid evolution of gas followed by resolidification. The residue of 1-phenylpyrazolo[3,4-d]pyrimidine-4-one (1-phenylallopurinol) (93c) formed needles, m.p. 298-299°C, in almost quantitative yield from water. (Found: C, 62.5; H, 3.81; N, 26.3; M⁺, 212; C₁₃H₁₀N₄O requires C, 62.26; H, 3.77; N, 26.4%; M, 212), Rf 0.7, identical with an authentic sample (mixed m.p., t.l.c., i.r., u.v.) prepared below.

b) Cheng and Robins route: - 5-Amino-1-phenylpyrazole-4-carbox-
amide (92c) (15 g) was heated with formamide (50 ml) at 190-200° for 30 min. The cooled solution was diluted with water (50 ml) and allowed to stand in the refrigerator overnight. The product was then filtered and washed with water, and recrystallised from water to yield small needles (11 g), m.p. 299°, of the 1-phenylpyrazolo[3,4-d]pyrimidine-4-one (93c) which proved to be identical (mixed m.p., i.r., u.v.) with the specimen prepared in (a).

5-Cyano-2-ethylthio-4-oxo-1,3-thiazine (72)

Cyanoacetic acid (8.5 g) and ethyl dithiocarbamate (12.1 g) were shaken with acetic anhydride (11 ml) at 25° for 60 h. The resulting precipitate was filtered off, washed with a little ether (2X30 ml) and extracted (Soxhlet) with ether (150 ml) for 18 h. Ethyl N-cyanoacetyldithiocarbamate (333) (5.7 g) crystallised from the extract as yellow needles, m.p. 140-142°, and recrystallised from methanol had m.p. 142°. Evaporation of the original filtrate in vacuo gave ethyl N-acetyldithiocarbamate (334) (5.7 g) which separated from water as yellow needles, m.p. and mixed m.p. 124°.

The dithiocarbamate (333) (20 g), triethyl orthoformate (28 g) and acetic anhydride (40 ml) were boiled under reflux for 1 h. The red solution, when cooled, gave crystals which were filtered off and washed with a little ice-cold ethyl acetate. 5-Cyano-2-ethylthio-4-oxo-1,3-thiazine (72) (8.5 g) separated from benzene/carbon tetrachloride as orange yellow leaflets, m.p. 140°. The filtrate was evaporated and the residue boiled under reflux for 1 h, when the solution was cooled, a further quantity of the thiazine (4 g) separated. The crude thiazine obtained in these experiments was pure enough for most purposes.
Ethyl N-(α-cyano-β-phenylhydrazinoacryloyl)dithiocarbamate (336)

Solutions of 5-cyano-2-ethylthio-4-oxo-1,3-thiazine (72) (1.98 g) in ethanol (30 ml) and phenyl hydrazine (1.1 ml) in ethanol (10 ml) were mixed at 0° and allowed to warm to room temperature slowly. A crystalline precipitate gradually appeared. The dithiocarbamate (336) (1.4 g) formed pale yellow needles, m.p. 140° (decomp-resolidified and then had m.p. > 300°. (Found: C, 50.8; H, 4.65; N, 18.15; C13H14N4OS requires: C, 50.98; H, 4.58; N, 18.3%)

The compound had no coloured derivative in the Bratton Marshall test and had diffuse u.v. absorption from 225 to 275 nm, Rf 0.95.

5-Amino-1-phenylpyrazole-4-(N-ethylthiothiocarbonyl)carboxamide (337)

When the foregoing dithiocarbamate was crystallised from hot nitromethane or when the foregoing reaction was carried out at temperatures >5°, the pyrazole (337) was produced. It had no CN absorption, gave a positive Bratton Marshall test (λ max 514 nm) and had u.v. absorption maxima at 312 and 267 nm. It separated from nitromethane as needles, m.p. 140° (decomp. then resolidified and had m.p. > 300°. (Found: C, 51.05; H, 4.55; N, 18.3; C13H14N4OS requires: C, 50.98; H, 4.58; N, 18.3%)

4-Hydroxy-6-mercapto-1-phenylpyrazolo[3,4-d]pyrimidine (332)

Attempt 1. From the acryloyldithiocarbamate (336): The foregoing dithiocarbamate (336) (1 g) was heated at 140° under nitrogen for 10 min. The sample melted with evolution of ethanthiol then resolidified. The mercaptopyrazolopyrimidine (332) (0.7 g) crystallised from ethanol (charcoal) as needles, m.p. > 300°. (Found: C, 54.0; H, 3.35; N, 23.0 M, 244. C13H14N4OS requires:
C, 54.1; H, 3.28; N, 22.95% M, 244) u.v. absorption at $\lambda_{\text{max}}$ 313 and 254 nm (pH 7) and $\lambda_{\text{max}}$ at 318 and 235 nm (pH 10), $R_f$ 0.8. If the reaction was carried out in the absence of nitrogen, an amorphous yellow solid was obtained with u.v. absorption at $\lambda_{\text{max}}$ 285 and 262 nm (no change in alkali), similar to values for 6-chloro-4-hydroxy-1-phenylpyrazolo[3,4-d]pyrimidine (97c), suggesting that the compound might be the disulphide of the foregoing mercaptopyrazolo pyrimidine (338).

**Confirmation of the structure:**

a) The foregoing mercaptopyrazolopyrimidine (332) (0.1 g) was boiled with water (10 ml) containing chloroacetic acid (0.14 g) for 4.5 h, the u.v. spectrum of the solution was then identical with that of 1-phenylpyrazolo-[3,4-d]pyrimidine-4,6-dione (94c) ($\lambda_{\text{max}}$ 288 nm). The solution was evaporated and cooled to give the pyrazolopyrimidine (94c) (0.07 g), m.p. $>$ 300. (Found: C, 58.25; H, 3.39; N, 24.7 M, 228; C$_n$H$_s$N$_t$O$_r$ requires C, 57.89; H, 3.51; N, 24.56% M, 228), identical (mixed m.p., i.r.) with an authentic sample.

b) The foregoing mercaptopyrazolopyrimidine (332) (1.5 g) was boiled in ethanol (50 ml) with Raney nickel (11 g wet) for 4 h. The solution was evaporated and the residue crystallised from water to give 1-phenylpyrazolo[3,4-d]pyrimidine-4-one (93c) (1.3 g) (Found: M, 212; Calc. for C$_n$H$_s$N$_t$O$_r$ M, 212), identical with an authentic sample prepared as outlined earlier.

**Attempt 2.** By reaction of 6-chloro-4-hydroxy-1-phenylpyrazolo-[3,4-d]pyrimidine (97c) with thiourea: A mixture of 1-phenylpyrazolo[3,4-d]pyrimidine-4,6-dione (94c) (40 g), phosphorous pentachloride (160 g) and phosphoryl chloride (500 ml) was boiled under reflux for 2 h. The excess phosphoryl chloride was removed
by evaporation and the syrupy residue poured with vigorous stirring, onto crushed ice (1 kg). The solution was extracted with chloroform, and the chloroform extract was washed with water and dried. Evaporation of the solvent gave 4,6-dichloro-1-phenyl-pyrazolo[3,4-d]pyrimidine (96c) (42 g) as a yellow solid, m.p. 120-122, which gave white needles, m.p. 126-127, from n-heptane.

A solution of the foregoing dichloro compound (96c) (5 g) in water (100 ml) containing potassium hydroxide (5 g) and activated charcoal (1 g) was boiled under reflux for 3 h. The solution was filtered and the hot filtrate acidified with acetic acid. The precipitate was collected and reprecipitated to give white needles (4.5 g), m.p. 280-281, of 6-chloro-1-phenyl-pyrazolo[3,4-d]pyrimidine-4-one (97c).

A mixture of the pyrazolopyrimidine (97c) (2.5 g) and thiourea (1.5 g) in ethanol (50 ml) was boiled under reflux for 5 h. From this mixture the starting material was recovered unchanged.

An intimate mixture of the pyrazolopyrimidine (97c) (2 g) and thiourea (1.5 g) was heated at 210° for 2 h. From this mixture the pyrimidine was recovered unchanged.

**Attempt 3. By direct fusion of 5-amino-1-phenylpyrazole-4-carboxamide (92c) and thiourea.**—An intimate mixture of the carboxamide (92c) (5.5 g) and thiourea (11 g) was maintained at 210° for 2 h. The final mixture was dissolved in potassium hydroxide solution. This solution, on acidification with acetic acid gave the original carboxamide unchanged.

4,6-Dichloro-1-methylpyrazolo[3,4-d]pyrimidine (96b)

4,6-Dihydroxy-1-methylpyrazolo[3,4-d]pyrimidine (94b) (100 g) was mixed with phosphoryl chloride (200 ml) and phosphorous penta-
chloride (700 g). The mixture was boiled under reflux for 28 h and the excess phosphoryl chloride was removed by distillation under reduced pressure. The syrupy residue was poured, with vigorous stirring, onto crushed ice (2 kg). The cold, aqueous suspension was filtered and the filtrate extracted with chloroform. The chloroform extract, after being washed well with ice water until free of acid, was dried over anhydrous magnesium sulphate. The solution was then evaporated to dryness and the residue solidified on cooling to give the required dichloro compound (96b) as a tan solid, m.p. 82-85°. The product was recrystallised from absolute ethanol to give white needles, m.p. 87-88°.

6-Chloro-4-hydroxy-1-methylpyrazolo[3,4-d]pyrimidine(97b)

The 4,6-dichloro derivative (96b) (5 g) was boiled under reflux with a mixture of potassium hydroxide (5 g) and activated charcoal (1 g) in water (100 ml) for 3 h. The solution was filtered and the hot filtrate acidified with acetic acid. The precipitate was collected and reprecipitated to give white needles, m.p. 267-268° (4 g) of the pyrazolopyrimidine (97b).

4-Hydroxy-6-mercapto-1-methylpyrazolo[3,4-d]pyrimidine(341)

1) From the foregoing chloropyrazolopyrimidine (97b) :- A mixture of 6-chloro-4-hydroxy-1-methylpyrazolo[3,4-d]pyrimidine (47b) (5.5 g) and thiourea (3 g) was boiled under reflux in ethanol (100 ml) for 5 h. The solid product was filtered and reprecipitated from dilute potassium hydroxide solution by acetic acid, to give a white solid, m.p. >300° (4.0 g) (λ max 247 and 275 nm).

2) From 5-amino-1-methylpyrazole-4-carboxamide (92b) :- The pyrazole (92b) (55 g) was fused with thiourea (100 g) at 210° for 2 h. The fused product was dissolved in potassium hydroxide solution
followed by reprecipitation with acetic acid. This process was repeated twice to give a white solid, m.p. >300° (41 g) (λ max 247 and 275 nm).

3) From the thiazine(72):—Solutions of 5-cyano-2-ethylthio-4-oxo-1,3-thiazine(72) (1.98 g) in nitromethane (20 ml) and methylhydrazine (0.48 g) in nitromethane (10 ml) were mixed at 5°. An immediate yellow colouration was observed and a yellow solid precipitated overnight (1 g), ν max 2230 cm⁻¹ (CN), which gave a positive Bratton Marshall test (λ max 504 nm). Recrystallisation of this material from nitromethane gave 5-amino-1-methylpyrazole-4-(N-ethylthiothiocarbonyl)carboxamide(340) as yellow needles. (No CN absorption and strong Bratton Marshall test (λ max 504 nm)).

The original precipitate was maintained at 250-280 under a nitrogen atmosphere for 20 min, during which time the solid melted, effervescence occurred and the gas, when passed through aqueous alkaline potassium permanganate solution, gave a precipitate of manganese dioxide. The material resolidified.
A small amount of sublimate was isolated (λ max 237 and 270 nm) as white needles, composition unknown. The solid residue was dissolved in aqueous potassium hydroxide solution and reprecipitated with acetic acid to give the thiopyrazolopyrimidine(341) as a white solid, m.p. >300° (λ max 247 and 275 nm). (Found: C, 39.2; H, 3.5; N, 31.1; M⁺ 182; C₆H₆N₅OS requires C, 39.56; H, 3.3; N, 30.77%; M, 182). This preparation was found to be identical to the compound made by methods 1 and 2, on the basis of identical ultraviolet spectra, melting points and mixed melting points.
1-Methylallopurinol (93b)

1) By reduction of the foregoing pyrazolopyrimidine (341):- A mixture of 4-hydroxy-6-mercapto-1-methylpyrazolo[3,4-d]pyrimidine (341) (0.1 g), prepared by route 3 above, and Raney nickel type 101 (1 g wet weight), in ethanol (20 ml) was boiled under reflux. The reaction was followed by ultraviolet spectroscopic studies and after 3.5 h the spectrum had changed to that of 1-methylallopurinol prepared below by the methods of Cheng and Robins.

2) By fusion of 5-amino-1-methylpyrazole-4-carboxamide (92b) and formamide:- A solution of the pyrazole (92b) (4 g) in formamide (10 ml), was boiled gently under reflux for 2 h. An equal volume of water was added to the cooled solution, which was set aside in a refrigerator overnight and then was filtered. The crude product was purified by dissolution in hot, dilute potassium hydroxide solution with reprecipitation by neutralising with acetic acid. Recrystallisation from water gave the required methylallopurinol (3.6 g) as white crystals, m.p. >300°.

4-Hydroxy-6-mercapto[3,4-d]pyrimidine (344)

1) By fusion of 5-aminopyrazole-4-carboxamide (92a) and thiourea:- A mixture of the pyrazole (92a) (5 g) and thiourea (10 g) was fused by heating at 160° for 20 min. The clear solution went mushy and heating was continued at 190° for a further 20 min, until the mixture became solid. This solid was dissolved in hot aqueous sodium hydroxide, from which the mercapto[pyrazolopyrimidine (344) was obtained as a white solid (2.4 g), m.p. >300° by neutralisation of the solution with acetic acid.

2) From the thiazine (72):- To a solution of 5-cyano-2-ethylthio-4-oxo-1,3-thiazine (72) (1.98 g) in nitromethane (20 ml)
was added dropwise an emulsion of hydrazine (0.5 ml) in nitromethane (10 ml) at 0. An immediate yellow precipitate was obtained (0.85 g) (i.r. absorption at \( \nu_{\text{max}} 2230 \text{ cm}^{-1} \) (CN), Bratton Marshall test \( \lambda_{\text{max}} 508 \text{ nm} \)). This crude solid was heated at 150-190\(^{\circ} \), under a nitrogen atmosphere, for 15 min, during which time the solid partially melted and then resolidified. Recrystallisation of this solid proved to be very difficult, in each case an oil was produced.

The solvents used were nitromethane, ethanol, dioxan, D.M.S.O., and sodium hydroxide and acetic acid. Separation of the crude solid by t.l.c. indicated that there were several components to the mixture, one of which corresponded to the sample prepared by route 1. Separation of the above mixture by column chromatography using a water, acetic acid, butanol mixture gave enough of the required component for direct u.v. comparison with an authentic sample, they were found to be identical. (\( \lambda_{\text{max}} 248 \) and 287 nm). 4-Hydroxy-6-mercaptopyrazolo[3,4-d]pyrimidine (344) was not prepared in any reasonable yield by this route.

\( \alpha \)-Cyano-\( \beta \)-ethoxy-N-benzoylacrylamide (351)

A mixture of cyanoacetic acid (14.8 g), benzamide (21 g) and acetic anhydride (23 ml) was heated on a water bath for 1.5 h. A brown solution was obtained which when cooled, gave a crystalline precipitate, which was filtered off and washed with ether. N-(Cyanoacetyl)benzamide (349) (7 g) separated from ethanol (charcoal) as needles, m.p. 184\(^{\circ} \).

A mixture of the foregoing benzamide (349) (6.9 g), triethyl orthoformate (5.7 g) and acetic anhydride (8 g) was boiled under reflux for 1 h. The crystals which separated on cooling were filtered off and washed with a little ethanol.
\( \alpha \)-Cyano-\( \beta \)-ethoxy-N-benzoylacrylamide(351) (5 g) crystallised from ethanol as needles, m.p. 143°.

\( \alpha \)-Cyano-\( \beta \)-ethoxy-N-acetylacrylamide(292)

A mixture of cyanoacetic acid (17 g), acetamide (11.8 g) and acetic anhydride (37 ml) was heated on a water bath for 3 h. Cooling gave a crystalline precipitate, which gave N-acetyl-\( \alpha \)-cyanoacetamide(350) (8 g) from ethanol (charcoal) as colourless plates, m.p. 156°.

A solution of the foregoing acetamide(350) (3.1 g) in triethyl orthoformate (3.6 g) and acetic anhydride (5 g) was boiled under reflux for 1 h. The pale brown solution was evaporated to a small volume in vacuo and cooled. The crystalline precipitate of \( \alpha \)-cyano-\( \beta \)-ethoxy-N-acetylacrylamide(292) (2.8 g) separated from benzene or benzene/light petroleum as needles, m.p. 190°.

6-Phenyl-4-hydroxypyrazolo[3,4-d]pyrimidine(354)

To a solution of o-cyano-\( \alpha \)-ethoxy-N-benzoylacrylamide(351) (3.0 g) in ethanol (30 ml) was added hydrazine hydrate (1.0 g). An immediate pale yellow precipitate (1.7 g) (m.p. 152°, resolidified then 290°) was obtained, which was filtered off and washed with a cold ethanol/ether mixture. This sample of 5-aminopyrazole-4-(N-benzoyl)carboxamide(353) proved to be rather difficult to purify, and so was used directly to prepare the pyrazolopyrimidine. (Found: M⁺, 230 and 212; Calc. for C₃₃H₂₄N₆O₂: M⁺, 230) m/e, 212(M-H₂O)⁺. This compound had no CN absorption, and gave a coloured derivative with the Bratton Marshall reagents (\( \lambda \) max 535 nm).

A sample of the aminopyrazole(353) was heated to 170° and maintained at this temperature for about 10-15 min. The sample melted, gave off steam, and slowly resolidified. Recrystallisation...
from aqueous sodium hydroxide and acetic acid gave 6-phenyl-4-hydroxypyrazolo[3,4-d]pyrimidine (354), as a white powder and recrystallisation from nitromethane gave the pyrimidine as needles, m.p. 294-296°, u.v. λ max 252 nm. (Found: C, 62.3; H, 3.61; N, 26.2; M, 212; C_{12}H_{11}N_{4}O requires: C, 62.26; H, 3.77; N, 26.42%; M, 212). If the original reaction was performed at 0° the precipitate obtained possessed i.r. absorption at λ max 2210 cm^{-1}(CN), due to the presence of the acyclic intermediate (352).

6-Phenyl-4-hydroxy-1-methylpyrazolo[3,4-d]pyrimidine (357)

To a solution of α-cyano-β-ethoxy-N-benzoylacrylamide (351) (3.0 g) in warm alcohol (35 ml) was added methylhydrazine (0.9 g). On standing overnight, yellow needles were obtained which were filtered off and washed with a cold ethanol/ether mixture. Recrystallisation of this material from nitromethane gave 5-amino-1-methylpyrazole-4-(N-benzoyl)carboxamide (356) as colourless needles (2.1 g), m.p. 204° (effervesced, resolidified, then 275°). This compound possessed no CN absorption but gave a highly coloured derivative with the Bratton Marshall reagents. (λ max 530 nm). (Found: M^{+}, 244; Calc. for C_{12}H_{11}N_{4}O : M, 244) m/e, 227(M-OH)^{+}, 226(M-H_{2}O)^{+}. A sample of the aminopyrazole (356) was maintained at 200-220° for 10 min. The sample melted at 200°, bubbled and partially resolidified when gaseous evolution had ceased. The cooled mass was recrystallised from nitromethane giving 6-phenyl-4-hydroxy-1-methylpyrazolo[3,4-d]pyrimidine (357) as needles, m.p. 280°. (λ max 271 nm). (Found: M^{+}, 226, Calc. for C_{12}H_{11}N_{4}O : M, 226) m/e 149(M-C_{6}H_{5})^{+}.

6-Phenyl-4-hydroxy-1-phenylpyrazolo[3,4-d]pyrimidine (360)

To a solution of α-cyano-β-ethoxy-N-benzoylacrylamide (351)
(3.0 g) in warm alcohol (30 ml) was added phenylhydrazine (1.8 g). A yellow precipitate formed overnight, which was filtered off, washed with a cold ethanol/ether mixture and recrystallised from nitromethane, to give 5-amino-1-phenylpyrazole-4-(N-benzoyl)-carboxamide(359) as plates, m.p. 159° (resolidified then 268°). This material gave no CN absorption but gave a positive Bratton Marshall test (λ max 514 nm). This experiment was repeated at 5° and the precipitate obtained absorbed strongly in the i.r. at v max 2210 cm⁻¹(CN), indicating the presence of the acyclic intermediate, α-cyano-β-phenylhydrazino-N-benzoyl-acrylamide(358). Recrystallisation of (358) from nitromethane gave needles, identical to those prepared above, of the pyrazole (359). (Found: M⁺, 306; Calc. for C₁₄H₁₆N₄O₂: M⁺, 306) m/e 288(M-H₂O)⁺.

A sample of the aminopyrazole(359) was heated slowly to 200° and maintained at this temperature for 20 min. The sample melted at 150°, bubbled and slowly resolidified. This cooled product was recrystallised from nitromethane and gave 6-phenyl-4-hydroxy-1-phenylpyrazolo[3,4-d]pyrimidine(360) as needles, m.p. 275°. (Found: C, 71.2; H, 3.81; N, 19.1; M⁺, 288; C₁₁H₁₀N₂O requires: C, 70.83; H, 4.17; N, 19.44 %; M, 288) (u.v. absorption λ max 263 nm).

6-Methyl-4-hydroxy pyrazolo[3,4-d]pyrimidine(363)

a) From the acetyl reagent(292) :- To a solution of α-cyano-β-ethoxy-N-acetylacrylamide(292) (3.6 g) in methanol (30 ml) was added hydrazine hydrate (1.3 g). The resulting pale brown precipitate, m.p. 115° (resolidified then) 300°, was collected and washed with a cold methanol/ether mixture. This material possessed no CN absorption and gave a highly coloured product when diazotized and coupled with the Bratton Marshall reagents (λ max 520 nm).
(Found: M+ 168; Calc. for C6H9N4O2: M, 168) m/e 150(M-H2O); 135, (M-H2O, -Me).

A small sample of the above material was heated in an oil bath to 170-180° and maintained at this temperature. The sample melted at 115° and steam was evolved. After 15 min, gaseous evolution had stopped and a sticky solid was left. Recrystallisation of this solid from nitromethane (charcoal) gave 6-methyl-4-hydroxypyrazolo[3,4-d]pyrimidine (363) as a crystalline solid, m.p. > 300°. This sample gave no coloured product with the Bratton Marshall reagents but absorbed in the u.v. at \( \lambda_{\text{max}} \) 259 nm (pH 11) and \( \lambda_{\text{max}} \) 252 nm (pH 1), and was found to be identical (m.p., mixed m.p., t.l.c., u.v.) to a sample prepared below using the methods of Cheng and Robins.

b) Cheng and Robins: 5-Amino-4-cyanopyrazole (91a): Ethoxymethylenemalononitrile (150 g) was carefully added, in small portions, to 85% hydrazine hydrate (100 g). After approximately one half of the ethoxymethylenemalononitrile had been added, the reaction mixture was cooled slightly in cold water and the remaining ethoxymethylenemalononitrile added at such a rate that the contents of the flask boiled gently upon the addition of each 3-5 g portion. After all the ethoxymethylenemalononitrile had been added, the reaction flask was heated for 1 h on the steam bath. To the solidified mass was added water (100 ml) and the solution was set aside in the refrigerator overnight. The mushy solution was then filtered and the solid washed with cold water (50 ml). The crude yield of the tan coloured material was 97 g, m.p. 169-170°. Recrystallisation from water gave a white crystalline material, m.p. 174-175°.
5-Acetylamino-4-cyanopyrazole(364)

A mixture of acetic anhydride (250 ml) and 5-amino-4-cyanopyrazole(91a) (80 g) was refluxed for 10 h. Excess acetic anhydride was distilled off under reduced pressure. The syrupy residue was poured into benzene (30 ml). The mixture was stirred for several minutes, and the product crystallised slowly. The solid was collected and recrystallised from water to give 5-acetylamino-4-cyanopyrazole(364) as white crystals, m.p. 214-218°.

6-Methyl-4-hydroxypyrazolo[3,4-d]pyrimidine(363)

A mixture of 5-acetylamino-4-cyanopyrazole(364) (1.5 g), 10 % aqueous potassium hydroxide solution (7 ml) and 3 % aqueous hydrogen peroxide solution (15 ml), was warmed on a water bath for 30 min. The temperature of the bath was kept at 70-75°. The mixture was then acidified with acetic acid. A white precipitate formed gradually from the clear solution. It was filtered and reprecipitated from dilute potassium hydroxide solution and acetic acid to give 6-methyl-4-hydroxypyrazolo[3,4-d]pyrimidine(363) as a white powder, m.p. >300°(1.1 g). (Found: C, 48.3; H, 3.98; N, 37.4; C<sub>6</sub>H<sub>9</sub>N<sub>2</sub>O requires : C, 48.0; H, 4.00; N, 37.3 %). This material was found to be identical (m.p., mixed m.p., t.l.c., u.v.) to the above sample prepared by reaction of the acetyl reagent and hydrazine.

6-Methyl-4-hydroxy-1-methylpyrazolo[3,4-d]pyrimidine(368)

a) From the acetyl reagent and methylhydrazine:- To a solution of α-cyano-β-ethoxy-N-acetylacrylamide(292) (3.6 g) in methanol (30 ml) at room temperature, was added methylhydrazine (1.1 g). The resulting dark coloured solution gave a crystalline material overnight, m.p. 210°(decomp), which was
found to possess weak i.r. absorption at 2230 cm⁻¹ (CN), indicating the presence of a small quantity of the acyclic intermediate, α-cyano-β-methylhydrazino-N-acetylacrylamide (366). Recrystallisation of the above material from nitromethane gave 5-amino-1-methylpyrazole-4-(N-acetyl)carboxamide (367) as plates, m.p. 212⁰ (decomp), which gave a coloured product with the Bratton Marshall reagents (λ max 509 nm). (Found: M, 182; Calc. for C₉H₁₀N₂O₂: M, 182); m/e, 164(M - H₂O)⁺, 139(M - MeCO)⁺, 124(M - MeCONH)⁺, 123(M - MeCO, -NH₂)⁺.

A small sample of the above pyrazole (0.5 g) was heated carefully in an oil bath. It melted at 230⁰ and the temperature was maintained at 230-240⁰ for 10 min. During this time, steam was evolved and the material resolidified. Recrystallisation of the fused product from nitromethane (charcoal) gave 6-methyl-4-hydroxy-1-methylpyrazolo[3,4-d]pyrimidine (368), as short needles, m.p. 281⁰. (Found: M, 164; Calc. for C₇H₆N₂O: M, 164); m/e 149(M - Me)⁺ (u.v. absorption λ max 268 nm (pH 11) and λ max 253 nm (pH 1)). (368) was found to be identical (m.p., mixed m.p., t.l.c., u.v.) to a sample of the required pyrazolo[3,4-d]-pyrimidine prepared below by the method devised by Cheng and Robins.

b) Cheng and Robins route: 5-Acetamino-4-cyano-1-methylpyrazole (369):- A mixture of acetic anhydride (250 ml) and 5-amino-4-cyano-1-methylpyrazole (91b) (80 g) was heated under reflux for 10 h. Excess acetic anhydride was distilled off under reduced pressure. The resulting syrup was stirred into benzene (30 ml), and after a few min, the product crystallised and was collected and recrystallised from water to give the
required pyrazole(369), m.p. 210-211\(^\circ\), as a white crystalline powder (90 g).

6-Methyl-4-hydroxy-1-methylpyrazolo[3,4-d]pyrimidine(368)

5-Acetylamino-1-methyl-4-cyanopyrazole(369)(1.21 g) was added to a mixture of 3 % aqueous hydrogen peroxide (15 ml) and 10 % aqueous potassium hydroxide (4 ml). The mixture was warmed at 70\(^\circ\) for 10 h. It was then filtered and the filtrate acidified to yield a light yellow precipitate. The crude product gave 6-methyl-4-hydroxy-1-methylpyrazolo[3,4-d]pyrimidine(368) as needles, m.p. 278-280\(^\circ\), from ethanol. (Found: C, 51.2; H, 4.88; N, 34.2; C\(_9\)H\(_7\)N\(_4\)O requires: C, 51.22; H, 4.88; N, 34.15 %).

6-Methyl-4-hydroxy-1-phenylpyrazolo[3,4-d]pyrimidine(372)

To a solution of \(\alpha\)-cyano-\(\beta\)-ethoxy-N-acetylacrylamide(292) (3.6 g) in methanol (30 ml) was added phenylhydrazine (2.2 g). The resulting dark coloured solution was allowed to stand overnight. Pale brown crystals were produced, which gave 5-amino-1-phenyl-4-(N-acetyl)acrylamide(371) as needles, m.p. 210\(^\circ\) (decomp), which gave a highly coloured product with the Bratton Marshall reagents (\(\lambda_{max}\) 527 nm). (Found: M, 244; Calc. for C\(_9\)H\(_8\)N\(_2\)O : M, 244); m/e, 226(M - H\(_2\)O), 185(M - MeCO,NH). A small sample of the pyrazole(371) (0.5 g) was heated in an oil bath to 220-230 and maintained at this temperature for about 5 min. During this time the sample melted, evolved steam and resolidified, in the form of crude needles. Recrystallisation of the solid from nitromethane gave 6-methyl-4-hydroxy-1-phenylpyrazolo[3,4-d]pyrimidine(372) as long needles, m.p. 295\(^\circ\). (Found: M, 226; Calc. for C\(_9\)H\(_8\)N\(_2\)O : M, 226); m/e, 211(M - Me), (u.v. absorption \(\lambda_{max}\) 275 nm (pH 11) and \(\lambda_{max}\) 231 nm (pH 1)).
**Ethyl N-(α-cyano-β-ethoxy-N-methylacryloyl)carbamate(67)**

**Route 1. Ethyl N-methylcarbamate:** A solution of 25-30% aqueous methylamine (726 ml; 675 g) in diethyl ether (1200 ml) in a 5 litre mechanically stirred flask, was cooled in an ice/salt bath at below 5 ° and maintained at this temperature throughout the experiment. Ethyl chloroformate (770 ml; 868 g) was added in portions so as to keep the temperature below 5 °. After the addition of about half of the ethyl chloroformate a cold aqueous solution of sodium hydroxide (320 g in 480 ml) was added, along with the ethyl chloroformate, so that the additions ended together. The total time for the addition of the ethyl chloroformate was 4.5 h and the mixture was allowed to stand for a further 0.5 h. The ether layer was removed, and the aqueous layer was extracted with ether (400 ml). The ether extracts were combined, dried over anhydrous potassium carbonate and evaporated to low volume. From this ethyl N-methylcarbamate (600 g) was obtained by vacuum distillation, using a water pump, at 65-75 °, as a colourless oil.

**Ethyl N-methyl-N-(cyanoacetyl)carbamate(376):** A mixture of cyanoacetic acid (172 g), ethyl N-methylcarbamate (208.8 g) and acetic anhydride (408 g), (the acid and anhydride being initially mixed and warmed to allow easy formation of the mixed anhydride, acetylcyanoacetate(377)) were warmed together on a water bath at 70 ° for 3 h. The slightly cooled solution was poured into water (3000 ml) and the precipitated oil was extracted with ether (3 X 1000 ml). The combined ether extract was neutralised with aqueous sodium bicarbonate, water washed to remove excess bicarbonate, dried over anhydrous sodium sulphate and evaporated to low volume, leaving an oil (193 g). This oil was distilled
under vacuum. The first fraction (50 g) was collected at 80-100°
(at a pressure of 21 mm of mercury), and contained mostly ethyl N-
methylacetylcarbamate. Ethyl N-methyl-N-(cyanoacetyl)carbamate
(376) was collected in the second fraction (109.6 g) at 120°
(at a pressure of 0.5 mm of mercury), as a colourless oil.

A mixture of ethyl N-methyl-N-(cyanoacetyl)carbamate (104 g),
triethyl orthoformate (104 g) and acetic anhydride (200 ml) was
heated under reflux for 1 h. The mixture was distilled under
vacuum, ethyl N-(α-cyano-β-ethoxy-N-methylacryloyl)carbamate(67)
was collected as a fraction distilling at 150° (pressure 0.1 mm
of mercury). (Found: C, 52.8; H, 6.28; N, 12.5; M, 226; \( \text{C}_7\text{H}_8\text{N}_2\text{O}_4 \)
requires: C, 53.1; H, 6.19; N, 12.39 %, M, 226).

Route 2: To a solution of ethyl N-(cyanoacetyl)carbamate
(379) (1 g) in dry ether, was added, in portions, a solution of
diazomethane in dry ether. Nitrogen was evolved and the green
colouration of the diazomethane solution faded. Eventually gaseous
evolution ceased and a permanent green colour remained. The solution
was evaporated to low volume and the resulting oil (0.7 g) was
distilled under vacuum. The fraction collected at 120 (0.5 mm
of mercury), was found to be identical to the ethyl N-methyl-N-
(cyanoacetyl)carbamate prepared by route (1), and its reaction
with triethyl orthoformate gave a product identical with the
ethyl N-(α-cyano-β-ethoxy-N-methylacryloyl)carbamate(67)
prepared above.

Route 3: To a stirred suspension of ethyl N-(α-cyano-β-
ethoxyacryloyl)carbamate(124) (3 g) in dry ether (30 ml) was added,
in portions, a solution of diazomethane in dry ether. Nitrogen
was evolved, the green colour of the diazomethane solution faded
and the suspension slowly dissolved. When all reaction had stopped
and the solution was a permanent green colour, it was evaporated
to low volume leaving a yellow oil. This oil proved to be unreactive
to ammonia, methylamine and phenylethylamine. (Found: M, 240 ;
Calc. for C_{n}H_{n}N_{n}O_{n} :M, 226 ; Calc. for C_{n}H_{n}N_{n}O_{n} : (M +CH): 240).

5-Cyano-3-methyl-1-anilinouracil(386): To a solution of
ethyl N-(α-cyano-β-ethoxy-N-methylacryloyl)carbamate(67) (2.2 g)
in alcohol (8 ml) at room temperature, was added phenylhydrazine
(1.5 g). After stirring for 2 min, a precipitate was produced
which was washed free of the highly coloured mother liquors with
cold alcohol. The pale yellow solid (2.0 g), m.p. 210°, on
recrystallisation from alcohol, gave white needles, m.p. 220°,
which failed to give a coloured product with the Bratton Marshall
reagents. This solid was deduced to be 5-cyano-3-methyl-1-anilino-
uracil. (Found: C, 59.27; H, 4.03; N, 23.05; M, 242; C_{n}H_{n}N_{n}O_{n}
requires: C, 59.5; H, 4.13; N, 23.14 %; M, 242), λmax (pH 7),
274 and 218 nm; λmax (pH 10), 283 and 212 nm; λmax (pH 1), 278
and 219 nm. I.r. spectrum showed a strong peak at ν max 2235 cm^{-1}(CN).

5-Cyano-3-methyl-1-aminouracil(388): - To a solution of ethyl-
N-(α-cyano-β-ethoxy-N-methylacryloyl)carbamate(67) (2.2 g) in
alcohol (10 ml), was added hydrazine hydrate (0.8 g). Considerable
heat was generated and the yellow solution was set aside overnight.
Chunky yellow crystals(387) (1.9 g), m.p. 140°, were produced,
which were collected, ether washed and tested with the Bratton
Marshall reagents. A very pale pink coloured derivative was
formed. (Found for the yellow crystals: M, 212; Calc. for
C_{n}H_{n}N_{n}O_{n} : M, 212); m/e, 166(M - EtOH). I.r. showed strong absorption
at ν max 2240 cm^{-1}(CN).
A sample of the above ethyl N-\((\alpha\text{-cyano-}\beta\text{-hydrazino-N-methyl-}
\text{acryloyl})\)carbamate(387) was heated to 160 and maintained at this
temperature for 5 min. The solid melted, effervesced and resolid-
ified. This solid gave 5-cyano-3-methyl-1-aminouracil(388) as
chunky white crystals, m.p. 220°, from nitromethane. (Found: M, 166;
Calc. for C\textsubscript{4}H\textsubscript{7}N\textsubscript{4}O\textsubscript{2}: M, 166). I.r. showed strong absorption at
\(\nu_{\text{max}} 2240 \text{ cm}^{-1}(\text{CN})\).

5-Cyano-3-methyl-1-methylaminouracil(391) :- To a solution
of ethyl N-\((\alpha\text{-cyano-}\beta\text{-ethoxy-N-methylacryloyl})\)carbamate(67) (2.2 g)
in alcohol (8 ml) was added methylhydrazine (0.5 g). Considerable
heat was generated by the reaction and the resulting green coloured
solution, when cooled, gave a yellow oil which crystallised upon
scratching. The solid was collected (1.3 g) and recrystallised
from nitromethane, giving 5-cyano-3-methyl-1-methylaminouracil
(391) as needles, m.p.201. (Found: M, 180; Calc. for C\textsubscript{7}H\textsubscript{8}N\textsubscript{4}O\textsubscript{2}:
M, 180).I.r. showed a strong absorption peak at \(\nu_{\text{max}} 2238 \text{ cm}^{-1}(\text{CN})\).

L-Rhamnopyranosylhydrazone(403)

L-Rhamnose (25 g) was added in portions to an ice cooled
mixture of absolute methanol (80 ml) and hydrazine hydrate (65 ml).
The sugar dissolved and a small amount of white precipitate was
produced, which redissolved on standing overnight at room tempera-
ture. The solution was evaporated, to leave an oil, which was
mixed with absolute ethanol (75 ml) and re-evaporated. This process
was repeated two more times. The resulting oil was taken up in
methanol (100 ml) from which crystals of L-rhamnopyranosylhydrazone
(14.5 g), m.p. 120°, were obtained by cooling and scratching.

D-Galactopyranosylhydrazone(404)

D-Galactose (35 g) was added in portions to an ice cold mixture
of 100% hydrazine hydrate (60 ml) and methanol (75 ml). The solution was set aside at room temperature, and an initial heavy white precipitate slowly redissolved after about 10 h. After 24 h, the solution was filtered to remove small traces of the original precipitate, and evaporated to low volume under vacuum. To the resulting oil was added ethanol (75 ml) and the mixture was re-evaporated. This process was repeated three more times. The resulting oil was triturated under absolute methanol (120 ml) and a solid formed (15.2 g), m.p. 116-122°. The mother liquors, on standing, gave a second crop of crystals of D-galactopyranosyl-hydrazone (404) (5.3 g), m.p. 118-121°, identical (mixed m.p., t.l.c) to the first crop of crystalline material.

D-Ribopyranosylhydrazone (405)

D-Ribose (21 g) was added in portions to a cooled mixture of hydrazine hydrate (60 ml) and absolute methanol (75 ml). An initial precipitate dissolved on standing for 10 h. The pale yellow solution was kept at room temperature for 24 h, and then evaporated to low volume under vacuum. The oil was dried over phosphorous pentoxide at 0.1 mm Hg pressure for 6 h. The resulting thick viscous syrup was redissolved in methanol (100 ml) which on dilution with absolute ethanol (100 ml), precipitated a gummy oil. The mother liquor was decanted off and methanol (50 ml) was added to the oil. Trituration failed to induce crystallisation. The oil, containing D-ribopyranosylhydrazone, despite being repeatedly mixed with methanol and ethanol and re-evaporated, failed to crystallise and so was used as an oil.

D-Xylopyranosylhydrazone (406)

D-Xylose (80 g) was added in portions to a stirred ice cold
mixture of 100% hydrazine hydrate (200 ml) and methanol (250 ml). A solution was formed which was allowed to stand overnight, then evaporated to low volume. To this oil was added methanol (150 ml) and the mixture was re-evaporated. This process was repeated four more times. The resulting colourless oil was triturated under methanol (150 ml) and most of the oil dissolved. The addition of ether (20 ml) precipitated the hydrazone as an oil. The mixture was again evaporated to low volume and the resulting oil was taken up in methanol (100 ml). To this solution was added a small quantity of solid carbon dioxide (drycold) which rapidly cooled the solution, precipitating D-xylopyranosylhydrazone (406) as a white powder (43 g), m.p. 110-114, which when collected quickly, remained solid. If the solution was allowed to warm up, then the white powder changed back into an oil.

D-Glucopyranosylhydrazone (407)

D-Glucose (10 g) was suspended in a mixture of methanol (40 ml) and 100% hydrazine hydrate (9 g). The suspension slowly dissolved and an oil was precipitated. After 5 h, no suspension remained and the precipitated oil was hard and toffee like. The oil was collected, mixed with methanol (25 ml) and the mixture was evaporated. This process was repeated two more times, however, the hydrazone (407) could not be induced to crystallise. It was therefore used as an oil.

D-Arabinopyranosylhydrazone (408)

D-Arabinose (6.7 g) was added in portions to an ice cold mixture of hydrazine hydrate (20 ml) and methanol (25 ml). The solution was set aside in a refrigerator for 20 h, kept at room temperature for 18 h, and evaporated to low volume in a vacuum
dessicator (0.5 - 1.0 mm Hg pressure). This evaporation required approximately two days. To the resulting oil was added methanol (25 ml) and a solid white precipitate of D-arabinopyranosylhydrazone (408) (3.2 g), m.p. 108-113, was obtained, which was collected and dried under vacuum in a dessicator.

5-Cyano-3-methyl-1-L-rhamnopyranosylaminouracil (410)

L-Rhamnopyranosylhydrazone (403) (2 g) was stirred into acetonitrile (10 ml). Ethyl N-(α-cyano-β-ethoxy-N-methylacryloyl)carbamate (67) (2.5 g) was added to the stirred mixture and a green solution was produced, into which the hydrazone slowly dissolved with warming. By cooling this solution, the acyclic intermediate ethyl N-(α-cyano-β-L-rhamnopyranosylhydrazino-N-methylacryloyl)carbamate (409) was recovered as a white powder, m.p. 105° (bubbled and resolidified to melt again at 206°). I.r. indicated strong absorption at $\nu_{\text{max}}$ 2230 cm$^{-1}$ (CN). When the original reaction mixture was heated under reflux for 2 h, a precipitate was produced which gave 5-cyano-3-methyl-1-L-rhamnopyranosylaminouracil (410) as needles, m.p. 206° (decomp). (Found: C, 46.22; H, 5.30; N, 17.81; C$_{11}$H$_{14}$N$_2$O$_2$ requires : C, 46.15; H, 5.13; N, 17.95 %) U.v. showed strong absorption at $\lambda_{\text{max}}$ (pH 7) 279 and 223 nm; $\lambda_{\text{max}}$ (pH 1), 276 and 221 nm; $\lambda_{\text{max}}$ (pH 10), 281 and 223 nm. I.r. showed strong absorption at $\nu_{\text{max}}$ 2230 cm$^{-1}$ (CN).

5-Cyano-3-methyl-1-D-ribopyranosylaminouracil (412)

The oil containing D-ribopyranosylhydrazone (405) (1.65 g) was triturated under acetonitrile (40 ml). Some of the oil appeared to dissolve. Ethyl N-(α-cyano-β-ethoxy-N-methylacryloyl)carbamate (67) (2.5 g) was added. A green colouration was produced in the solution which slowly darkened as more hydrazone dissolved. The
mixture was heated under reflux for 4 h, a small quantity of
undissolved solid was removed and the solution was set aside
overnight at room temperature. 5-Cyano-3-methyl-1-D-ribopyranosyl-
aminouracil was isolated as a pale green chunky crystalline
material which gave chunky crystals, m.p. 175° (decomp), from
nitromethane. (Found: M⁺, 298; Calc. for C₁₁H₁₇N₂O₅; M, 298) (i.r.
showed strong absorption at νmax 2242 cm⁻¹ (CN) and the u.v. spectrum
indicated absorption peaks at λmax (pH 10), 283 and 221 nm and
λmax (pH 7), 275 and 220 nm).

5-Cyano-3-methyl-1-D-galactopyranosylaminouracil

To a stirred suspension of D-galactopyranosylhydrazone (1.94 g) in acetonitrile (30 ml) was added ethyl N-(o-cyano-ß-
ethoxy-N-methylacryloyl)carbamate (2.5 g). The mixture was
heated under reflux for 4 h, during which time the hydrazone
dissolved, leaving a green solution and a small amount of a soft
white solid. This solid was recovered and found to be crude D-
galactopyranosylhydrazone (m.p. and i.r.). On standing overnight
at room temperature, a rather sticky crystalline material, m.p.
143°, was produced, which when collected and recrystallised from
water gave, after considerable time, 5-cyano-3-methyl-1-D-galacto-
pyranosylaminouracil as a white crystalline solid, m.p. 168°
(decomp). (I.r. showed strong absorption at νmax 2230 cm⁻¹ (CN)
and u.v. absorption peaks were found at λmax (pH 10), 279 and 228 nm;
λmax (pH 7), 274 and 220 nm).

Ethyl N-(α-cyano-β-L-rhamnopyranosylhydrazinoacryloyl)carbamate

To a solution of ethyl N-(α-cyano-β-ethoxyacryloyl)carbamate
(3.1 g) (recrystallised prior to use from ethyl acetate)
in isopropanol (40 ml) was added L-rhamnopyranosylhydrazone (403)
(2.5 g) (washed with methanol and ether till hydrazine free). The hydrazone slowly dissolved over a period of 3 days to be replaced by a flocculent white precipitate. During the reaction the solution attained a pale green colouration which later faded. The precipitate was collected and the gummy solid, on trituration under ether gave ethyl N-((α-cyano-β-L-rhamnopyranosylhydrazinosacryloyl)-carbamate(414) as a white amorphous powder (3.4 g), m.p. 118-123° (decomp). I.r. absorption at \( \nu_{\text{max}} \) 2250 cm\(^{-1}\) (CN), u.v. \( \lambda_{\text{max}} \) (pH 7), 287 nm.

5-Amino-1-L-rhamnopyranosylpyrazole-4-(N-ethoxycarbonyl)carboxamide(415)

ai) From the acyclic carbamate(414) with heat :- A small sample of the above carbamate(414), on heating to 80-90° for a few min, gave 5-amino-1-L-rhamnopyranosylpyrazole-4-(N-ethoxycarbonyl)-carboxamide(415), as an amorphous solid indistinguishable in appearance from the starting material. (415) showed no absorption band characteristic of (CN) in its i.r. spectrum however, and its u.v. spectrum showed absorption at \( \lambda_{\text{max}} \) (pH 7) 280nm; \( \lambda_{\text{max}} \) (pH 10), 273 nm and \( \lambda_{\text{max}} \) (pH 1), 277 nm. A u.v. spectrum identical to the above was obtained by the neutralisation of an alcoholic solution of the carbamate(414), which had been treated with a small quantity of sodium hydroxide solution.

a ii) From (414) with sodium hydroxide :- To an aqueous solution of the carbamate(414) (1g in 10 ml) was added 0.5 M sodium hydroxide solution (5 ml). The solution was immediately neutralised with Dowex 50W-X8 (H\(^{+}\)) resin which was removed. The solution on evaporation, cooling and scratching, failed to give a crystalline material, so was evaporated to dryness as a solid foam, m.p. 105-120°, identical (i.r. and u.v.) to the sample obtained by heating (414).
b) One stage preparations: 1) To a solution of ethyl N-(α-cyano-β-ethoxyacryloyl)carbamate (124) (6.2 g) in isopropanol (25 ml) was added L-rhamnopyranosylhydrazone (403) (2.5 g). The mixture was heated under reflux until all solid had dissolved (15 min). The resulting green coloured solution was maintained at this temperature for a further 10 min, cooled and scratched. No crystalline material being produced, the solution was reduced in volume, cooled and scratched. The solution again failed to yield a crystalline deposit. Finally the solution was evaporated to dryness and the resulting solid foam, m.p. 110-122°C, was found to possess u.v. and i.r. absorption spectra identical to those samples of 5-amino-1-L-rhamnopyranosylpyrazole-4-(N-ethoxycarbonyl)-carboxamide (415) prepared above.

2) A non-crystalline sample of (415) was also obtained by reaction of the hydrazone (403) (2.5 g) and the acrylamide reagent (124) (3.5 g) in dioxan (10 ml). A solution was obtained by stirring overnight at room temperature, which on dilution with ether (7 ml), precipitated an oil. The supernate liquor was decanted off and ether (10 ml) was added to the oil. On stirring an amorphous solid was produced (4 g; 85%), m.p. 115-120°C, which was found to be identical (u.v. and i.r.) to the above samples of (415). The above experiment was repeated using water, acetonitrile, dimethylformamide and nitromethane as solvent. In each case the amorphous product was identical (i.r. and u.v.) to (415) prepared above. All samples of (415) readily gave a coloured derivative (λ max 510 nm) with the Bratton Marshall reagents.

1) By fusion of 5-amino-1-L-rhamnopyranosylpyrazole-4-(N-
-ethoxycarbonyl)carboxamide(415) : - Four samples of (415) (each 0.5 g) were heated and maintained at a certain temperature for a specified period of time. Comparison was made of the u.v. spectrum of each sample, both before and heating. Sample a) was maintained at 100-110° for 10 min, the u.v. spectra indicated no change in the structure. Sample b) was maintained at 130-140° for 10 min, the sample melted, however the u.v. spectra indicated no change in the structure. Sample c) was maintained at 150-160° for 5 min. The sample melted and evolved gas for about 3 min after which gaseous evolution ceased. The u.v. spectra indicated a change in structure, initial absorption at λmax 276 nm moved to final absorption at λmax 248 nm. Some darkening of the material occurred. On cooling, the material solidified as an amorphous material. Sample d) was maintained at 160-200° for 10 min, and behaved in a manner exactly similar to sample c) except that the final material appeared to be more extensively degraded. The spectra showed absorption peaks identical to those recorded for sample c) except that the final spectrum of d) was less clearly defined. A comparison of their i.r. spectra confirmed the similarity of the products from samples c) and d).

The fusion products were purified by dissolution in hot water (charcoal). Evaporation of the resulting pale green solution gave 1-L-rhamnopyranosylpyrazolo[3,4-d]pyrimidine-4,6-dione(416) as a solid foam, m.p. 134-140°.

2) Treatment of (415) with aqueous sodium hydroxide solution : - To a solution of the pyrazole(415) (0.5 g) in water (5 ml) was added 2M aqueous sodium hydroxide (5 ml). This solution was heated under reflux for 1h, samples were taken at regular intervals and
analysed for u.v. absorption. The initial absorption peaks at 
$\lambda_{\text{max}}$ 273 nm progressively faded and a new absorption peak at $\lambda_{\text{max}}$
247 nm (identical to the absorption of the fused samples c) and 
d) above) developed, leaving a single absorption peak after about
9 min. No further changes occurred in the u.v. spectrum during the
remainder of the experiment, however, the colour of the reaction
solution gradually darkened. This experiment was repeated, and
terminated when the u.v. spectrum failed to show absorption at
$\lambda_{\text{max}}$ 273 nm. The solution was cooled, neutralised with Dowex
50W-X8 (H$^+$) resin, filtered, evaporated and cooled. No crystalline
material was produced so the solution was evaporated to dryness
and gave the pyrazolopyrimidine(416) as a solid pale green foam,
m.p. 135-140, identical (mixed m.p., i.r. u.v.) to the purified
fused samples prepared above.

The above experiment was repeated varying the solution
temperature. No cyclisation occurred at temperatures below 60°, and
at 60° cyclisation required at least 2 h. At temperatures above 60°,
progressively less time was required to achieve complete cyclisation.

3) Heating the pyrazole(415) in methyl cellosolve :- A sample
of the pyrazole(415) (0.5 g) was heated under reflux in methyl
cellosolve (10 ml). Samples were taken at regular intervals and
the progress of the reaction was followed by u.v. studies. The
u.v. absorption peak at $\lambda_{\text{max}}$ 277 nm slowly faded and the peak
at $\lambda_{\text{max}}$ 248 nm slowly developed, the whole process required about
4 h. The final solution was very dark in colour and was therefore
treated with activated charcoal, which removed some of the
discolouration. Evaporation and cooling of this solution failed
to yield any crystalline material therefore the solution was
evaporated to dryness and gave the pyrazolopyrimidine (416) (0.4 g) as a solid foam, identical (i.r. and u.v.) to the samples prepared above.

4) Cyclisation of (415) with sodium ethoxide: - To a solution of the pyrazole (415) (1 g) in isopropanol (10 ml) was added an alcoholic solution of sodium ethoxide (10 ml) (2 g per 100 ml). An immediate yellow precipitate was formed, which was assumed to be the sodium salt of the pyrazole. This was collected and washed free of sodium ethoxide with methanol. The precipitate gave a strong yellow flame test, confirming the presence of sodium. The precipitate was dissolved in water (10 ml) and neutralised with M sulphuric acid. On evaporation and cooling a crystalline material was produced, which was isolated and shown to be sodium sulphate. Apart from this no crystalline material was recovered. The mother liquors showed absorption (u.v. and i.r.) identical to the starting material (415).

Purification of 1-L-rhamnopyranosylpyrazolo[3,4-d]pyrimidine-4,6-dione (416)

A sample of the pyrazolopyrimidine (416) (3 g), which was found to give a coloured derivative (λ max 510 nm) when treated with the Bratton Marshall reagents, was extracted with boiling ethanol. The ethanolic extract gave a highly coloured derivative with the above reagents (λ max 510 nm) and the derivative given by the residue from the sample of (416), was less highly coloured. The solid material was therefore extracted with ethanol (45 ml) using a Soxhlet apparatus for 3 h. The insoluble material recovered gave no coloured derivative with the Bratton Marshall reagents, but absorbed strongly in the u.v. at λ max 247 nm.
A column of Amberlite I.R. 120 (NH₃ form) resin was prepared by washing Amberlite I.R. 120 (Na⁺ form) resin with M sulphuric acid to remove the sodium ions, 2 M ammonium hydroxide to remove the acid, and water to remove excess alkali. To this column was added a solution of the above alcohol treated sample of (416) in water (5 ml). The column was eluted with water and fractions were collected, rich in the compound absorbing at λ max 247 nm (assumed to be the pyrazolopyrimidine(416)). These fractions were evaporated and recrystallisation of the resulting foam was attempted, to no avail. (Found: M⁺ 151; Calc. for C₇H₇N₂O₂; M, 298; m/e 151(base fragment, C₅H₇N₂O₂⁺), 150(base fragment - H), 147(sugar fragment, C₅H₇O₂⁺).

5-Amino-1-D-glucopyranosylpyrazole-4-(ethoxycarbonyl)carboxamide (418)

To a solution of ethyl N-(α-cyano-α-ethoxyacryloyl)carbamate (124) (2.4 g) in isopropanol (30 ml) was added D-glucopyranosyl-hydrazone (407) (2 g) (gum). The mixture was heated under reflux for about 1.5 h. During this time the gum dissolved and a green coloured solution was produced, which on evaporation to dryness, gave 5-amino-1-D-glucopyranosylpyrazole-4-(ethoxycarbonyl)carboxamide (418) as a solid foam (4 g), m.p. 150-156. (U.v. λ max (pH 7), 283 and 218 nm; λ max (pH 1), 281 and 218 nm and λ max (pH 10), 276 and 224 nm). Diazotisation of (418) and coupling with the Bratton Marshall reagents gave a highly coloured derivative (λ max 521 nm). A small sample of the original, unheated reaction solution, maintained at room temperature for 2 days, gave i.r. absorption at ν max 2210 cm⁻¹(CN), showing the presence of the acyclic carbamate (417).

1-D-Glucopyranosylpyrazolo[3,4-d]pyrimidine-4,6-dione (419)

The above solid foam (418) (4 g) was dissolved in an aqueous
solution of 2 M sodium hydroxide (10 ml) and the resulting solution was heated under reflux for 20 min. A change in the u.v. spectrum of the sample was observed during the course of the reaction from λmax 276 nm to λmax 248 nm. The solution was cooled, neutralised with Dowex 50W-X8 (H⁺) resin, filtered and evaporated to dryness to give 1-D-glucopyranosylpyrazolo[3,4-d]pyrimidine-4,6-dione (419) as a solid foam (3.5 g), m.p. 163-167. A solution of this foam in water (5 ml) was placed on a column of Amberlite 120 (NH₃ type) resin, prepared by washing a column of Amberlite 120 (Na⁺ type) resin with M sulphuric acid, 2M ammonium hydroxide and water. The column was eluted with water and fractions were collected, rich in the compound absorbing at λmax 248 nm. These were evaporated to dryness to give (419) as a pale yellow foam, m.p. 165-168, attempted recrystallisation of which failed to give a crystalline product.

5-Amino-1-D-xylopyranosylpyrazole-4-(ethoxycarbonyl)carboxamide (421)

To a solution of ethyl N-(α-cyano-β-ethoxyacryloyl)carbamate (124) (9.5 g) in isopropanol (40 ml) was added D-xylopyranosyl-hydrazone (406) (7.5 g) and the mixture was heated under reflux. A yellow precipitate was produced after about 20 min and heating was continued for a further 40 min. The precipitate (4 g), m.p. 308, was collected and found to be insoluble in water. The filtrate was divided into two parts. One portion was evaporated to dryness to give 5-amino-1-D-xylopyranosylpyrazole-4-(ethoxycarbonyl)-carboxamide (421), as a solid foam, m.p. 140-150. To the other portion was added an alcoholic solution of sodium ethoxide (15 ml) (2 g per 100 ml). An immediate non-crystalline precipitate of the sodium salt (yellow flame test) of the pyrazole (421) was obtained
which was dissolved in water (15 ml). The solution was neutralised with Dowex 50W-X8 (H⁺) resin, filtered and evaporated to give a solid foam, m.p. 140-150, very similar (m.p., u.v.) to the foam (421) obtained above. (U.v. λmax (pH 7 and 1), 281 and 218 nm and λmax (pH 10), 273 nm). Diazotisation of (421) and coupling with the Bratton-Marshall reagents gave a coloured derivative (λmax 518 nm).

A small sample of the original, unheated reaction solution, maintained at room temperature for 2 days, gave i.r. absorption at νmax 2260 cm⁻¹ (CN) and u.v. absorption at λmax (pH 7), 287 nm, assumed to be the acyclic acryloylcarbamate (420).

5-Amino-1-D-arabinopyranosylpyrazole-4-(ethoxycarbonyl)carboxamide (422)

To a solution of ethyl N-(α-cyano-β-ethoxyacryloyl)carbamate (12h) (1.06 g) in dimethylsulphoxide (2 ml), at room temperature, was added D-arabinopyranosylhydrazone (408) (0.82 g). The hydrazone slowly dissolved (15 h) to leave a green solution (weak absorption at νmax 2275 cm⁻¹ (CN) ) which was heated on a steam bath for 1 h. The solution was cooled, diluted with ether (1 ml) and precipitated an oil which, when cooled and scratched, failed to solidify. The oil was taken up in ethanol and evaporated to dryness to give 5-amino-1-D-arabinopyranosylpyrazole-4-(ethoxycarbonyl)carboxamide (422) as a solid foam, m.p. 80-100. (U.v. λmax (pH 7 and 1), 281 and 220 nm and λmax (pH 10), 277 and 220 nm). Diazotisation of (422) and coupling with the Bratton-Marshall reagents gave a coloured derivative (λmax 525 nm).
REFERENCES

1. Brugratelli, Giornoledi Fisica, Chemica e Storia Naturate (Pavia), Decada Seconda, 1818, 1, 117.
5. Grimaux, Compt., Rend., 1878, 87, 752.
30. Grimaux, Ber., 1879, 12, 378.
33. Lund, Ber., 1936, 69, 1621.
34. Traube, Ber., 1900, 33, 1371.
39. Traube, Ann., 1904, 331, 64.
42. Foldi and Salamon, Ber., 1941, 74, 1126.
52. Ger. Pats., 171,992; 225,457; 179,946; 183,628.


82. Hsu, T.C., Robins, R.K., and Cheng, C.C., Science, 1956, 123, 848.


90. Shaw, R.K., Shulman, R.N. and Davidson, J.D., Roll, D.P. and Frei, E., Cancer, 1960, 13, 482.


106. Claisen and Haase, Ber., 1895, 28, 36.


192. B.P. Appl., 58,090 ; 1968, (to Wellcome Foundation).