BRANCHED-CHAIN CARBOHYDRATES
AND NUCLEOSIDES

by

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B.Sc. (Honours), University of British Columbia, 1972

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY
in
THE FACULTY OF GRADUATE STUDIES
In the Department of
Chemistry

We accept this thesis as conforming to the
required standard

THE UNIVERSITY OF BRITISH COLUMBIA
September, 1978
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Date March 14, 1979.
The syntheses of a number of branched-chain carbohydrates and nucleosides are reported.

The application of the Kiliani-Fischer cyanohydrin synthesis to 1,2:5,6-di-0-isopropylidene-α-D-ribo-hexofuranos-3-ulose (25) yielded either the 3-C-cyano-1,2:5,6-di-0-isopropylidene-α-D-gluco or allofuranose (26 and 27, respectively) predominantly, depending upon the conditions employed. The stereoselectivity of the reaction was examined. Reduction of the cyano group and acetylation of the resulting amine afforded 3-C-acetamido-methyl-1,2:5,6-di-0-isopropylidene-α-D-allo and gluco-furanose (53 and 54, respectively) and provided an unequivocal proof of structure for the original cyanohydrins.

The ylid generated by the reaction of potassium t-butoxide and carbomethoxymethylidimethyl phosphonate was condensed with 1,2:5,6-di-0-isopropylidene-α-D-ribo-hexofuranos-3-ulose (25) to afford, after reduction of the α,β-unsaturated esters (135 and 136), 3-C-(carbomethoxy-methyl)-3-deoxy-1,2:5,6-di-0-isopropylidene-α-D-allofuranose (35). The 5,6-0-isopropylidene group of 35 was hydrolyzed,
the resulting diol was oxidatively cleaved and reduced, and the resulting alcohol was benzoylated to give 5-0-benzoyl-3-C-(carbomethoxymethyl)-3-deoxy-1,2-0-isopropylidene-\(\alpha\)-D-ribofuranose (172). The 1,2-0-isopropylidene group of 172 was hydrolyzed to yield the 5-0-benzoyl-3-C-carboxymethyl-3-deoxy-\(\alpha\),\(\beta\)-D-ribofuranosyl-2,3-\(\gamma\)-lactone (173) which was acetylated to afford 1-0-acetyl-5-0-benzoyl-3-C-carboxymethyl-3-deoxy-\(\beta\)-D-ribofuranosyl-2,3-\(\gamma\)-lactone (169). Compound 169 was fused directly with 2,6-dichloropurine to give 2,6-dichloro-9-[3'-C-(carboxymethyl-2',3'-\(\gamma\)-lactone)-3'-deoxy-\(\beta\) and \(\alpha\)-D-ribofuranosyl]purine (174 and 175, respectively) and reacted with \(N^6\)-benzoyl-\(N^6\), 9-bis(trimethylsilyl)adenine, in the presence of stannous chloride, to afford \(N^6\)-benzoyl-9-(3'-C-carboxymethyl-2',3'-\(\gamma\)-lactone-3'-deoxy-\(\beta\)-D-ribofuranosyl)adenine (176) and the \(\alpha\)-anomer 177. De-benzoylation of the \(\beta\)-nucleoside 176 yielded 9-[3'-C-(carboxymethyl-2',3'-\(\gamma\)-lactone)-3'-deoxy-\(\beta\)-D-ribofuranosyl]adenine (178) while the \(\alpha\)-nucleoside 177 afforded 9-[3'-C-carboxymethyl-3'-deoxy-\(\alpha\)-D-ribofuranosyl] adenine (181).

Condensation of methyl nitroacetate with 5-0-benzoyl-1,2-0-isopropylidene-\(\alpha\)-D-erythro-pentofuranos-3-ulose (182) and 5-0-benzyl-1,2-0-isopropylidene-\(\alpha\)-D-erythro-pentofuranos-3-ulose (194) in the presence of ammonium acetate, followed by immediate treatment with p-toluenesulphonic acid monohydrate in acetic anhydride, yielded (E) and (Z) 5-0-
benzoyl and 5-0-benzyl-3-deoxy-1,2-0-isopropylidene-3-C-nitro(methoxycarbonyl)methylene-a-D-erythro-pentofuranose, respectively (189 and 198). In a similar set of reactions with 1,2:5,6-di-0-isopropylidene-a-D-ribo-hexofuranos-3-ulose (25), the major product, obtained in good yield, was 3-0-acetyl-1,2:5,6-di-0-isopropylidene-3-C-[(R,S)nitro(methoxycarbonyl)methyl]-a-D-allofuranose (165).

The 5,6-0-isopropylidene group of compound 165 was hydrolyzed and the resulting diol was treated with p-toluenesulphonic acid monohydrate in acetic anhydride at elevated temperature to afford, after reduction with sodium cyanoborohydride, 5,6-di-0-acetyl-3-deoxy-1,2-0-isopropylidene-3-C-[(R,S)-nitro(methoxycarbonyl)methyl]-a-D-allofuranose (241). Acetolysis of compound 241 gave 1,2,5,6-tetra-0-acetyl-3-deoxy-3-C-[(R,S)-nitro(methoxycarbonyl)methyl]-a,β-D-allofuranose (243) which was used in an unsuccessful nucleoside synthesis with bis(trimethylsilyl)thymine.

Reduction of the nitro group of 3-0-acetyl-1,2:5,6-di-0-isopropylidene-3-C-[(R,S)-nitro(methoxycarbonyl)methyl]-a-D-allofuranose by hydrogenation over Raney nickel afford methyl L- and D-2-(1,2:5,6-di-0-isopropylidene-a-D-allofuranos-3-yl)glycinate (205 and 206, respectively). The major product, methyl L-glycinate 205, was acetylated and the 0-acetate and 5,6-0-isopropylidene groups were hydrolyzed to yield a compound which was successively treated with sodium periodate and sodium borohydride to give, after acetylation, methyl N-acetyl-L-2-(1,2-0-
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Trifluoroacetylation of methyl L-2-(1,2:5,6-di-0-isopropylidene-α-D-allofuranos-3-yl)glycinate (205), followed by successive replacement of the 5,6 and 1,2-0-isopropylidene acetals with 0-acetate blocking groups, afforded methyl L-2-(1,2,3,5,6-penta-0-acetyl-α,β-D-allofuranos-3-yl)-N-trifluoroacetyl glycinate (250). The attempted fusion of the bromo-sugar derivative of compound 250 with N\textsuperscript{6}-benzoyl-N\textsuperscript{6}-9-bis-(trimethylsilyl)adenine yielded 1,1'-anhydro-2,3,5,6-tetra-0-acetyl-3-C-(R)-methoxycarbonyl-1(R),1'(S)-N-trifluoroacetoepimo-α-D-allofuranose (252) whereas the silver trifluoromethylsulfonate catalyzed condensation of the same bromo-sugar with bis(trimethylsilyl)thymine afforded 1-[2',3',5',6'-tetra-0-acetyl-3'-C-(S)-N-trifluoroacetyl-carbomethoxy(amo)methyl]-β-D-allofuranosyl]thymine (257).
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ACKNOWLEDGEMENTS

The author wishes to express his gratitude to Dr. Alex Rosenthal for his encouragement and skillful guidance throughout the course of this work.

My thanks are extended to Dr. L.D. Hall, Dr. E. Piers, Dr. G.G.S. Dutton and the members of their research groups for their many helpful suggestions during my period at U.B.C.

I would also like to thank the members of Dr. Rosenthal's research group and in particular Don Baker, Colin Richards, Murray Ratcliffe, Kent Dooley, and Robert Dodd, for their invaluable practical assistance during our association.

To Anne, for her encouragement, assistance and patient understanding during the preparation of this thesis, I wish to express my deepest appreciation.

Finally, the financial support of the University of British Columbia (1973-1978), the H.R. MacMillan Family Scholarship Fund (1973-1975), and the National Research Council of Canada (1972-1973, 1975-1978) is acknowledged.
I. OBJECTIVE

Since the early 1950's the interest in synthesizing branched-chain carbohydrates and nucleosides has markedly increased owing to the finding that such modifications of naturally occurring compounds have resulted in interesting changes in their biological activity. In particular, a change of functionality or branching at C-3 position is observed in many such naturally occurring carbohydrates. The work described herein involves the introduction of substituents at this position.

The Kiliani-Fischer cyanohydrin synthesis has recently been applied to 1,2:5,6-di-O-isopropylidene-α-D-ribo-hexofuranos-3-ulose to yield either the 3-C-cyano-1,2:5,6-di-O-isopropylidene-α-D-gluco or alloburanose predominantly, depending on the conditions employed. Reduction of the cyano group thus provides a facile route to the corresponding 3-C-aminomethyl carbohydrates.

The objective of the first part of this work was:

1) to examine the influence of the pH of the reaction medium on the stereochemistry of the products obtained;
2) to examine the degree of stereoselectivity operative in the production of the epimeric cyanohydrins and;

3) to provide a proof of structure for the products obtained.

In the second part of this work two objectives were sought. The first objective was the efficient synthesis of L-0-acetyl-5-0-benzoyl-3-C-(carboxymethyl)-3-deoxy-β-β-D-ribofuranose-2,3-γ-lactone and the second was to use this lactone to synthesize 3'-C-carboxymethyl α and β-adenine nucleosides that would serve as analogues of the antibiotic puromycin.

A range of commercially important glycosyl amino acid nucleosides, known as the Polyoxins, has recently been the centre of much synthetic endeavour. These thirteen polyoxins all contain an α-L-amino acid residue at C-4' of a furanosyl pyrimidine and it seemed to us that incorporation of the amino acid at the C-3' position, rather than the C-4' position, might result in interesting biological consequences. The primary objective of the final portion of this work was, therefore, two-fold in nature. The first goal was the synthesis of a fully blocked derivative of L-2-(D-allofuranos-3-yl)glycine and the second goal was to use such a compound for the synthesis of 1-[(S)-3'-C-carboxy(amino)methyl-β-D-allofuranosyl]thymine. Such a thymine derivative would
serve as an analogue of the nucleoside moiety of the antibiotic polyoxin J. Towards this end, the condensation of methyl nitroacetate with various 3-ketofuranoses was investigated and as a result the synthesis of 3-deoxy-3-C-nitro(carbomethoxy)methyl furanoses was also investigated.

In order to provide some background for subsequent discussions, a brief summary will be made of the synthesis of branched-chain sugars, glycosyl amino acids and nucleosides.
II. INTRODUCTION

1. Branched-Chain Sugars

The enormous interest in branched-chain carbohydrates which has grown up in the past twenty years is largely the result of numerous reports concerning the isolation of such compounds from natural sources. Branched-chain sugars\textsuperscript{1,2} have been isolated from micro-organisms\textsuperscript{3,6}, higher plants\textsuperscript{7}, as components of cell wall polysaccharides\textsuperscript{8-11}, and it has been suggested that branched-chain sugars may also occur in man\textsuperscript{12}. Part of the reason for the increased interest in the synthesis of these compounds is undoubtedly due to the finding that nucleosides with branched-chain sugars can exhibit cytostatic or virostatic activity of possible therapeutic value\textsuperscript{13-15}.

Branched-chain sugars are divided into two types\textsuperscript{16}. Type A sugars are defined as those in which a hydrogen atom has been substituted by a group R while Type B sugars are defined as those in which a hydroxyl group has been substituted. Type B compounds are also defined as "deoxy" sugars. The first two reported branched-chain sugars \(\text{\textalpha}\)-apiose\textsuperscript{17,18} (1) and \(\text{\textbeta}\)-hamamelose\textsuperscript{19-20} (2) are represen-
tative of Type A sugars while \( L \)-garosamine\(^{21-22} \) (3) exemplifies a Type B sugar.

1.1 Synthesis of Branched-Chain Sugars

A very brief summary of some of the reactions applied to carbohydrates in order to introduce branching into the sugar skeleton will be discussed below. There have been several extensive reviews\(^1,2,23 \) on this subject and therefore only the most recent advances will be discussed. Many of the syntheses of branched-chain sugars involve condensation reactions with keto-sugars and these will be discussed in section 3. The condensation reactions which bear directly on the work described in this thesis will also be discussed in more detail in section 3.

1.1.1 Synthesis of Type A Sugars

Several methods have been employed to introduce branch-chains onto sugars while retaining a hydroxyl group at the branch-point.
Diazomethane has been reacted with keto-sugars to form an epoxide 4 which may then be opened with a variety of reagents. Thus the epoxide 4 may be treated with lithium aluminum hydride to give a C-methyl sugar (5a); alkali to give a C-hydroxymethyl sugar (5b); or ammonia to give a C-aminomethyl sugar (5c).

\[ \text{C} = \text{O} \xrightarrow{\text{CH}_2\text{N}_2} \text{C} = \text{O} \]

\[ \xrightarrow{\text{LiAlH}_4} \quad \xrightarrow{\text{OH}^-} \quad \xrightarrow{\text{NH}_3} \]

In a similar reaction the dimethylsulfoxonium methylid was condensed with the ketose 6 to afford an epoxide which was subsequently reduced to give the L-arabino product 7 (R=CH₃). Interestingly, the reaction of diazomethane with ketose 6 afforded, after lithium aluminum hydride reduction, the C-methyl sugar 8 (R=CH₃) which is the C-2 epimer of compound 7 (R=CH₃).³¹

Organolithium and organomagnesium compounds have been used extensively in the synthesis of epimeric Type A sugars. Thus treatment of the ketose 6 with organolithium
reagents affords the L-ribo sugar \(8^{28}\) whereas organo-
magnesium reagents yield predominantly the L-arabino
derivatives \(7^{27,29,30}\).

1.1.2 Synthesis of Type B Sugars

The application of the Wittig reaction to keto-sugars
has greatly increased the availability of type B sugars
and this reaction will be reviewed separately in
section 3.2.

Nucleophilic addition to anhydro-sugars has also
found considerable use in the synthesis of branched-chain
sugars\(^{32}\). Reaction of compound \(9\) with alkyllithium
reagents proceeded as expected with trans-diaxial cleavage
of the epoxide ring to give only the allo sugars \(10^{33}\).
Similarly other carbohydrate oxiranes have been opened
with cyanide\(^{34-36}\) and diethyl malonate carbanions\(^{37}\).

Improved methods for the synthesis of unsaturated
sugars\(^{38-44}\) have increased the use of photochemistry
in carbohydrate chemistry. The photoamidation of the unsaturated sugars 11, 12, and 13 to yield carbamoyl branched-chain and chain extended sugars 14-20 is an example of the use of photochemistry to afford both Type B\(^{45,46}\) and Type A\(^{47}\) sugars.
2. Methods of Oxidation

2.1 Oxidation of Secondary Hydroxyl Groups

The increase in the number of synthetic branched-chain sugars has paralleled the development of more effective and efficient methods of oxidizing secondary alcohols. In addition to the classical methods of oxidation which employ platinum oxide and oxygen, chromium trioxide-pyridine, or lead tetraacetate-pyridine, two reagents have largely dominated
the field of carbohydrate oxidations. These reagents are dimethyl sulfoxide (DMSO) and ruthenium tetroxide \((\text{RuO}_4)\). The methods of oxidation in carbohydrate chemistry have been thoroughly reviewed by Butterworth and Hanessian\(^{51}\) and therefore only the methods which were employed in this work (DMSO and \(\text{RuO}_4\)) will be discussed.

Since DMSO was first used as an oxidizing agent\(^{52}\) many variations have been successfully employed in which an activating electrophile (E) such as \(\text{N,N-dicyclohexyl-carbodiimide}\)\(^{53}\), acetic anhydride\(^{54}\) or phosphorus pentoxide\(^{55}\) is used in combination with DMSO. It is proposed\(^{56}\) that most DMSO oxidations involve the formation of a dimethyl-alkoxysulfonium intermediate \(21\) which is then displaced by the alcohol to form the sulfonium intermediate \(22\). The subsequent loss of a proton collapses the intermediate \(22\) to give a ketone \(23\) and dimethyl sulfide.

The first application of a ruthenium tetroxide oxidation to a carbohydrate was in 1964 when Overend and co-workers\(^{57}\) reported the oxidation of a 1,2:5,6-di-O-isopropylidene-\(\alpha\)-D-glucofuranose \((24)\) to give the keto-sugar \(25\), and since then it has been proved to be a powerful and useful reagent. Jones and co-workers\(^{58,59}\) have found that instead of adding a molar equivalent of the prepared tetroxide to the reaction medium, the tetroxide can be generated \textit{in situ} from a catalytic amount of
ruthenium dioxide\(^{60}\) and sodium or potassium periodate. As the tetroxide is consumed and converted back into the dioxide, more periodate is added and the cycle is continued.
until all of the alcohol has been oxidized. This is the manner in which ruthenium tetroxide oxidations are generally performed.

3. **Condensation Reactions Involving Keto and Aldehydo-Sugars**

The ready availability of keto and aldehydo-sugars has led to application of a myriad of condensation reactions to the synthesis of both Type A and Type B sugars. A discussion of all such reactions is beyond the scope of this work and only a brief review of the pertinent reactions will be given.

3.1 **Cyanide Condensations**

The use of cyanide ion as a nucleophile has been most widely applied to aldoses and is probably best known as the initial reaction of the Kiliani-Fischer synthesis in which the carbon chain of an aldose is lengthened by one carbon atom. The cyanohydrin synthesis has not been used very much to prepare branched-chain sugars from ketoses.

Tronchet and Bourgeois reported the synthesis of the allo-cyanohydrin by the reaction of potassium cyanide with 1,2:5,6-di-O-isopropylidene-α-D-ribo-hexofuranos-3-ulse (25). The product was assigned the allo structure in the belief that the cyanide ion would attack the carbonyl of the ketose from the less hindered
However, Bourgeois later reported that either the gluco or allo-cyanohydrin (26 and 27, respectively) could be synthesized exclusively depending upon the conditions employed. It was also pointed out that the cyanohydrin originally described by Tronchet was the thermodynamically more stable product and therefore the assignment of its configuration on mechanistic grounds was not valid.

The configuration of either cyanohydrin had not been established with any certainty until the completion of the research outlined herein.

3.2 The Wittig Reaction

Various compounds have been shown to afford phosphonium ylids if there exists a hydrogen atom adjacent to a phosphorus atom carrying a reasonable degree of positive charge as in the case of phosphoranes (28), phosphine oxides (29), phosphinates (30), and phosphonates (31). Of these, compounds 28 and 31 are the most commonly employed.
phosphonium ylids. Phosphorones 28 are used in the classical Wittig reactions while the phosphonates 31 are used in the modified Wittig reaction.

3.2.1 The Classical Wittig Reaction

Phosphoranes were used in condensation reactions as early as 1919 but it was not until thirty years later that their use developed into a widely applicable synthetic procedure.

In 1949 Wittig and Rieber treated tetramethylphosphonium iodide 32 with methyl lithium to give the methylenetrimethylphosphorane 33 which was then reacted with benzophenone to afford compound 34. In 1953 Wittig and Geissler reported the condensation of methylene-triphenylphosphorane with benzophenone to afford, in high yield, diphenylethylene. From that year the number of applications of the Wittig reaction multiplied greatly.
and both the mechanism and stereochemistry of the reaction were extensively investigated\textsuperscript{65,69-74}.

\[
\begin{align*}
(CH_3)_3 P-CH_3 I &+ CH_3 Li \rightarrow (CH_3)_3 P=CH_2 + LiI + CH_4 \\
(C_6 H_5)_2 C=O &\rightarrow (CH_3)_3 P-CH_2-C-(C_6 H_5)_2 OH
\end{align*}
\]

While the Wittig reaction has been used extensively in other areas its application in the carbohydrate field has been rather limited. Kochetkov and co-workers reported the reaction of carbethoxymethylenetriphenylphosphorane with aldoses, having either free\textsuperscript{75} or blocked\textsuperscript{76} hydroxyls, to afford $\alpha,\beta$-unsaturated aldonic acids. The double bond was dihydroxylated and the ester reduced to yield the expected chain-extended aldoses\textsuperscript{77}. In similar applications the Wittig reaction has also been used to prepare higher ketoses\textsuperscript{78}, aldonic acids\textsuperscript{79}, deoxy sugars\textsuperscript{80}, and C-glycosides\textsuperscript{81}. 
3.2.2 The Modified Wittig Reaction

In 1961, Wadworth and Emmons\textsuperscript{82} showed that stabilized phosphonate carbanions were more reactive than triarylphosphoranes towards some aldehydes and ketones. In addition they required milder conditions, were less expensive, and could be readily worked up in water since the resultant phosphate by-products were water soluble and did not cause the same problems in product isolation that triphenylphosphine oxide does in the classical Wittig reaction.

With this in mind, Rosenthal and Nguyen\textsuperscript{83} condensed the ylid formed by the reaction of potassium \( t \)-butoxide on carbomethoxymethylidimethylphosphonate with the ketosugar \textsuperscript{25} to yield, after hydrogenation, 3-\( C \)-(carbomethoxy-methyl)-3-deoxy-1,2:5,6-di-\( D \)-isopropylidene-\( D \)-allofuranose (\textsuperscript{35}). Similarly, reaction of the ylid formed from diethylcyanomethylphosphonate with compound \textsuperscript{25} afforded,
\[ \text{TrOCH}_2\text{O}_R \rightarrow \text{TrOCH}_2\text{O}_R \] 40 
\[ \begin{array}{c} \text{a) } R=\text{H}; R_1=\text{OMe} \\ \text{b) } R=\text{OMe}; R_1=\text{H} \end{array} \] 41 + 42
after hydrogenation, the cyanomethyl sugar \(36^{34,35}\).

Rosenthal, Catsoutacos, Richards, and Cliff applied the same reactions to the 2-deoxy-hexopyranos-3-ulose \(37\) and the 2-deoxy-pentofuranos-3-ulose \(40\) to afford the corresponding 3-C-(carbomethoxymethyl) sugar \(38^{36}\) and 3-C-(cyanomethyl) sugars \(39^{97}, 41^{48}, \text{and } 42^{88}\).

3.3 **Condensations Involving Nitro Compounds of the Type**

\[
\text{R-CH}_2\text{-NO}_2
\]

The condensation of nitromethane and its derivatives with aldehydo-sugars has long been investigated. As early as 1894 the nitromethane synthesis served as an alternative synthetic method for preparing higher-carbon aldoses by use of the Nef reaction \(89\). A route to the 2-deoxyaldoses was provided by applying the Nef reaction to unsaturated nitro sugars which were available from the acetylated nitro alditols by way of the Schmidt-Rutz \(90\) reaction. Some years later the "sugar dialdehydes" were elegantly cyclized with nitromethane to afford deoxynitro sugar intermediates \(91\).

The wide occurrence of amino sugars as constituents of antibiotics \(92,5\), and the discovery \(93\) of a naturally occurring nitro sugar (evernitrose) led to a number of investigations of the reaction of nitromethane with suitably blocked 3-keto-sugars.
3.3.1 Condensations Involving Nitromethane and 3-Keto Sugars

In the latter part of 1969 Lourens reported the condensation of nitromethane with two pentofuranos-3-uloses. When ketose 43 was treated with a suspension of 1.2 equivalents of sodium hydride in nitromethane the reaction yielded the ribo-nitromethyl sugar 44. Proof of the ribo configuration was provided by reducing compound 44 over palladium-on-charcoal and acetyling the product to afford compound 45. The tertiary hydroxyl of compound 44 was acetylated and the product 46 was subjected to standard Schmidt-Rutz reaction conditions to afford the nitro olefin 47. The double bond of compound 47 was then selectively reduced with sodium borohydride to yield the 3-deoxy-3-C-nitromethyl-D-ribo-furanose 48.

At approximately the same time Rosenthal, Ong and Baker reported the condensation of nitromethane with
the hexofuranos-3-ulo 25. When a solution of one equivalent of sodium methoxide was added to a solution of the ketose 25 in nitromethane the reaction yielded 1,2:5,6-di-0-isopropylidene-3-C-nitromethyl-α-D-glucofuranose (49).

The configuration at C-3 was determined by selectively hydrolyzing the 5,6-isopropylidene group, oxidizing the diol with sodium periodate, reducing the aldehyde with sodium borohydride and condensing the resulting product with acetone to give 1,2:3,5-di-0-isopropylidene-3-C-nitromethyl-α-D-xylofuranose (50). In the preliminary communication94 the initial condensation product was erroneously assigned the allo configuration (51) which was consistent with the reported steric control exerted by 1,2-0-isopropylidene groups96. It was subsequently found that a mixture of the gluco and allo compounds (4:1 of 49 and 51) was formed if the reaction was carried out in 1,2-dimethoxyethane and sodium hydride was used as
the base. Later, Moffatt and co-workers reported that the *allo* compound 51 was selectively produced by condensation of nitromethane and ketose 25 in anhydrous N,N-dimethylformamide using 0.1 equivalents of potassium t-butoxide. Dehydration of compound 51 in dimethylsulfoxide and acetic anhydride gave the nitro olefin 52 which, when subjected to base catalyzed hydration, yielded exclusively the *gluco* isomer 49.
Yoshimura et al.\textsuperscript{98} provided confirmation of the structures of the epimeric nitromethyl sugars by examining whether or not the corresponding 3-\textsubscript{C}-acetamidomethyl-1,2-\textsubscript{O}-isopropylidene-\textalpha-\textsubscript{D}-pentodialdofuranoses could form an aminal-ring between the aldehyde and acetamido group. Hydrogenation and acetylation of compounds 5\textsubscript{1} and 4\textsubscript{9} gave the corresponding 3-\textsubscript{C}-acetamidomethyl derivatives 5\textsubscript{3} and 5\textsubscript{4}, respectively. Hydrolysis of the 5,6-\textsubscript{O}-isopropylidene groups of compounds 5\textsubscript{3} and 5\textsubscript{4} and oxidation
of the products afforded the pentodialdofuranoses 55 and 56, respectively. The i.r. and p.m.r. spectra of 55 clearly showed that it existed as the aminal 55' while the spectra of compound 56 indicated the presence of an aldehyde. The configurations of the gluco-nitromethyl sugar 49 and the allo-nitromethyl sugar 51 were therefore unequivocally proven.

3.3.2 Condensations Involving Alkyl Nitroacetates With Aldehydo Sugars

The range of nitro compounds used in condensations with keto- and aldehydo-sugars has, until recently, been limited to nitroalkyl compounds. The use of alkyl nitroacetates in such condensations is very limited and has been restricted to aldehydo-sugars.

In 1969, Umezawa et al. 100 cyclized D'-methoxy-diglycolaldehyde (58), prepared by periodate cleavage of methyl β-L-arabinopyranoside (57), with ethyl nitroacetate to afford a mixture of cyclic α-nitro ester stereoisomers (59).
Catalytic reduction of the α-nitro esters, followed by chromatographic separation, gave four diastereomeric α-amino esters. Hydrolysis of each ester yielded the corresponding cyclic (+)-α-amino acid (60–63). The stereochemistry at C-2 and C-4 was determined by study of the n.m.r. spectra of the fully acetylated derivatives and by chemical degradation. In an analogous series of reactions α-D-xylopyranoside afforded three enantiomeric cyclic (−)-α-amino acids.

In 1973 Kornilov and co-workers\textsuperscript{101} condensed ethyl nitroacetate with isopropylidenated aldehydo-arabinose (64). When condensing agents such as triethylamine, piperidine, diethylamine, or butylamine were used the only product isolated was compound 66. It was proposed that such condensations proceed through the intermediate nitro olefin 65 to which another molecule of ethyl nitroacetate adds in a Michael-type reaction. The condensation of 64 with ethyl nitroacetate in 1:1 proportion proved to be possible.
only when ammonium acetate was used as the condensing agent in an aqueous-alcoholic medium. Under such conditions the nitro adduct 67 was produced in 50% yield. The acetylation
of compound 6\textsubscript{7} with acetic anhydride in pyridine produced the nitrolic ester 6\textsubscript{8} whereas, in the presence of catalytic amounts of sulphuric acid, the nitro group remained unaffected and the acetate 6\textsubscript{9} was the sole product.

4. **Nucleosides**

4.1 **Branched-Chain Nucleosides**

Branched-chain nucleoside is the term commonly used to describe a compound in which a nitrogen heterocycle, usually a purine or pyrimidine, is linked through nitrogen to the anomeric position of a carbohydrate which contains branching in its carbon skeleton.

In 1954 it was demonstrated by Baker and co-workers\textsuperscript{102} that 6-dimethylamino-9-(3'-amino-3'-deoxy-\beta-D-ribo-furanosyl)purine (70), obtained from the hydrolysis of puromycin, had biological activity that differed qualitatively and quantitatively from that of the antibiotic itself. This finding stimulated the synthesis of many other nucleosides modified at C-3' and other positions of the carbohydrate moiety and a number of these have been investigated for biological activity\textsuperscript{103,104}.

Excellent examples of branched-chain nucleosides possessing biological activity are the 2'-C and 3'-C methyl nucleosides (71-73)\textsuperscript{105} which have shown inhibition of KB cells\textsuperscript{106} in culture and are effective antivacinia agents in mice\textsuperscript{107}. The nitromethyl branched-chain nucleo-
70

71 \( R = H, R_1 = \text{Me} \)

72 \( R = \text{Me}, R_1 = H \)

73

74

75
sides 74 and 75 have recently been synthesized and shown to be active against KB tumor cells\textsuperscript{108}.

Many of the branched-chain nucleosides which possess biological activity contain peptide linkages and/or amino acid functionalities attached to the carbohydrate moiety. These compounds and their synthesis will be discussed in more detail in section 4.3.

### 4.2 Nucleoside Synthesis

There have been several reviews of the synthetic methods\textsuperscript{109,110} used in this greatly expanded field of carbohydrate chemistry and an excellent review\textsuperscript{111} of the mechanisms of these reactions has recently appeared. A discussion of all the various methods is beyond the scope of this thesis and therefore only the procedures used in the synthesis of compounds in the experimental section, and the background to these procedures, will be dealt with here.

#### 4.2.1 Fusion Reaction

The first application of Helferich's fusion reaction\textsuperscript{112} to the preparation of purine nucleosides was reported by Sato et al.\textsuperscript{113} in 1960. In this reaction tetra-0-acetyl-\(\beta\)-\(\alpha\)-D-ribofuranose was fused with various purines in the presence of a catalytic amount of \(p\)-toluenesulfonic acid under vacuum for 10-20 minutes.
Since these early reports a number of acids or Lewis acids, including p-toluenesulphonic acid\textsuperscript{114}, ZnCl\textsubscript{2}, TiCl\textsubscript{4}\textsuperscript{115}, and dichloroacetic acid\textsuperscript{116}, have been found to be effective catalysts for fusion reactions, while Ishido \textit{et al.}\textsuperscript{117,118} have demonstrated that with certain purines no acid catalyst is required.

In presenting a unified mechanism for the fusion of peracetylated sugars with purine bases, with or without catalysts, Watanabe, Hollenberg, and Fox\textsuperscript{111} made good use of the elegant studies of Ishido, Hosono \textit{et al.}\textsuperscript{117,118}.

After interpreting the kinetic data on the exclusive formation of 9-β-D-ribofuranosyl-2,6-dichloropurine (78) from the fusion of 2,6-dichloropurine (76) with β-D-ribofuranose tetracetate (77), without catalyst, Hosono \textit{et al.}\textsuperscript{118} proposed the following mechanism:
It had previously been noted\textsuperscript{117} that the acidity of the purine was also a factor in non-catalytic fusion reactions and it was suggested\textsuperscript{118} that the mechanism of the catalyzed and non-catalyzed reactions were similar except that "usual purines may be activated by interaction with acidic catalysts to react with fully acetylated sugars, and the corresponding nucleosides may be produced accompanied by an electron transfer such as depicted in the scheme" (see mechanism 77-78). Watanabe et al.\textsuperscript{111} expanded on this and proposed a mechanism similar to the trans-glycosylolation mechanism described\textsuperscript{111} for the heavy-metal procedure with purines.
In the case of a Lewis acid catalyst, such as ZnCl\(_2\), the mechanism proposed involves formation of a molecular complex between the purine and the catalyst with the Lewis acid attached to N-3 (79). This complex would be in equilibrium with an electronically imbalanced "activated" form of the purine (80). Intermediate 80 then attacks the acetylated sugar cation to form the N-9 nucleoside (82) with accompanying regeneration of the catalyst.

In the case of acid catalysts, the acid protonates the purine at N-3 to produce a purine cation analogous to 79. The conjugate base of the acid then abstracts the N-9 proton to form an "activated" purine (like 80) which is converted to the N-9 nucleoside 82 with liberation of the acid catalyst.

From the earliest reports\(^{113,119}\) the formation of anomeric mixtures was observed in fusion reactions. An illustrative example of the stereoselectivity of the reaction, with and without catalyst, is the fusion of 2,6-dichloropurine (76) with \(\alpha,\beta\)-ribofuranosyltetraacetate (85 and 77). Hosono et al. reported\(^{118}\) the following pertinent observations:

a) without catalyst the \(\beta\)-ribofuranosyltetraacetate 77 yielded the N-9-\(\beta\)-nucleoside exclusively, whereas the \(\alpha\)-ribofuranosyltetraacetate 85 afforded an anomeric mixture (\(\alpha,\beta=2:3\)).
b) with an acid catalyst, fusion of the β-sugar 77 with 76 for 1 minute gave only β-nucleoside, whereas fusion for 10 minutes yielded an α,β mixture.

c) treatment of the pure β-nucleoside with an acid catalyst at 150° for 10 minutes produced an anomeric mixture.

Watanabe rationalized these data by proposing the unified mechanism depicted below:
In the auto-catalytic fusion reaction of the β-acetate 77, attack of the C-1 acetoxy group on the purine proton would be anchimerically assisted by the 2-acetoxy group to produce a 1,2-acyloxonium ion 83 which would be attacked by the purine anion to yield the β-nucleoside 84. In the case of the α-sugar 85, assistance would come from the lone pair of electrons of the ring oxygen to produce the carboxonium ion 86. Such a carboxonium could rearrange to the cyclic acyloxonium ion 83 or react directly with the purine anion resulting in a mixture of anomers (84 and 87). The conversion of the β-nucleoside to the α-nucleoside under fusion conditions in the presence of an acid catalyst is explained by assuming that the protonated nucleoside 84 undergoes a reversal of the process to give sugar ions 83 and 86 and an "activated" purine analogous to compound 80.

4.2.2 The Hilbert-Johnson and Silyl Procedures

In 1930, Hilbert and Johnson 120 reacted 2,4-dimethoxy-pyrimidine (88, R=CH₃) with methyl iodide at room temperature to afford, after treatment with acid, 1-methyluracil. Later the same year, the scope of the reaction was extended by reaction of acetobromoglucose (89) with 2,4-diethoxy-pyrimidine (88, R=C₂H₅) to give the fully blocked nucleoside 90 121.
The mechanism of the Hilbert-Johnson reaction is generally depicted as follows:

The stereoselectivity of this reaction is analogous to that of the aforementioned fusion reaction in that even halogenoses containing a 2-acyloxy substituent produce anomeric mixtures of nucleosides. One of the mechanisms
proposed suggested that since most of the reported reactions involved anomeric mixtures of tri-0-acetyl-D-pentofuranosyl halides the products arose from a simple intermolecular $S_N^2$ displacement of the halide on C-1 by the N-1 of the base. This mechanism fails, however, to explain the production of anomeric mixtures when only one anomeric halide is employed.\textsuperscript{122,123}

It is now suggested\textsuperscript{111} that a mechanism analogous to that presented for the fusion reaction is operative.

In such a case "the relative contribution of carboxonium ion 98 to the overall reaction in effect would determine the relative amount of 1',2'-cis nucleoside in the product". When the acyloxonium ion 95 is excluded from the mechanism,
either by the use of 2-deoxy halogenoses or by blocking of the C-2 hydroxyl with a functionality incapable of participation, then one might expect α and β nucleosides to be produced in approximately equal amounts. Quite often, however, this is not the case and one isomer is formed predominantly. Some of the theories offered to explain such results have included:

a) the participation of an exocyclic 5-0-acyl substituent to form a 1,5-cyclic acyloxonium ion intermediate (100).\(^\text{123}\)

b) steric hinderance by 5-acyloxy substituents. It was concluded\(^\text{122,123}\) that this "steric effect" is more pronounced than the aforementioned "participation effect".

c) formation of a close ion pair\(^\text{111}\) (eg. 101) resulting in attack of the nucleophile from the side opposite to where the counter ion is located.

\[ R=\text{CH}_3, \text{Cl, NO}_2 \]

\[ R= (\text{C}_6\text{H}_5)\text{CH}_2 \]
The substitution of a trimethylsilyloxy functionality in place of the alkoxy group on the pyrimidine offered several advantages. Firstly, the lower electronegativity of silicon makes it more susceptible to nucleophilic attack and displacement and, secondly, the electron-releasing nature of the trimethylsilyloxy group increases the nucleophilicity of the corresponding pyrimidine.

The mechanism of nucleoside formation in the silyl modification is similar to that of the Hilbert-Johnson procedure (91→93) except that the leaving group (R) in 92 is a trimethylsilyl group instead of an alkyl group and the rate determining step is probably formation of the C-N bond instead of de-O-alkylation.

The stereochemistry of the silyl procedure also parallels that of the Hilbert-Johnson reaction in that mixtures of anomeric nucleosides are obtained even when halogenosides bearing a 2-acyloxy function are employed (see 94 and 97→96 and 99).

The application of the silyl modification to the synthesis of purine nucleosides bears many similarities to the fusion reactions discussed previously.

In this case the initial product is an N-3 glycosyl derivative which loses a trimethylsilyl group to form an electronically imbalanced species analogous to intermediate 80. Compound then undergoes an N-3 → N-9 transglycosylation to afford the final nucleoside.
product 105. Mixtures of anomeric nucleosides are sometimes obtained\textsuperscript{129} for the same reasons discussed for the fusion and Hilbert-Johnson reactions.

4.2.3 Catalyzed Hilbert-Johnson and Silyl Procedures

A number of catalysts have been used with the Hilbert-Johnson and silyl procedures in attempts to synthesize nucleosides in higher yields using milder conditions. To
date the catalysts have been of three general types - mercury salts, Friedel-Crafts catalysts, and derivatives of perfluoroalkane sulfonic and perchloric acids - and each will be discussed separately below.

4.2.3.1 Mercury Salt Catalysts

The addition of mercury salts to Hilbert-Johnson and silyl procedure reactions not only increases the rate of reaction but also influences the stereochemistry of the products.

Watanabe, Hollenberg, and Fox proposed a two-fold role for HgBr₂ in Hilbert-Johnson reactions. Firstly, that the mercury assists in the formation of the 1,2-acyloxonium ion (e.g. 95) from halogenoses bearing an α-2-acyloxy group thus favouring the formation of β-nucleosides and increasing the rate of reaction. In the case of 2-deoxy sugar halides (106), HgBr₂ assists in the formation of the carboxonium ion (107), thus increasing the rate of reaction and giving α/β product ratios closer to one. Secondly, they state, "the intermediate quaternary salt formed in the course of the reaction could complex with HgX₂ (structure 108) which would withdraw π electrons from the pyrimidine ring and enhance the cleavage of the 2-alkoxyalkyl group by halide ion". With mercury salts [i.e. HgBr₂, HgCl₂, Hg(OAc)₂ or HgO] the mechanism of the silyl procedure is proposed to be very similar to that of the Hilbert-Johnson
reaction. It may be formulated as follows:
4.2.3.2 Friedel-Crafts Catalysts

In 1970, Niedballa and Vorbrüggen\textsuperscript{134} showed that treatment of 2,4-bis-(trimethylsilyloxy)pyrimidines with per-acetylated sugars in the presence of Friedel-Crafts catalysts afforded N-1 pyrimidine nucleosides in good yields. Comparable results were obtained with a variety of catalysts including SnCl\textsubscript{4}, ZnCl\textsubscript{2}, TiCl\textsubscript{4}, BF\textsubscript{3}-ether, and AlCl\textsubscript{3} in 1,2-dichloroethane, acetonitrile, benzene, carbon-disulfide, or dimethylformamide. For reasons of experimental simplicity nearly all of the subsequent reports\textsuperscript{135-140} involved the use of 0.25 to 1.5 equivalents of SnCl\textsubscript{4} in 1,2-dichloromethane and/or acetonitrile. This reaction offers the advantage that 1-O-acetyl sugars\textsuperscript{134-140}, and in a few cases 1-O-methyl sugars\textsuperscript{134,135}, may be used directly instead of converting them into their more reactive and unstable sugar halides.

In the case of acylated 2-deoxy sugars\textsuperscript{135} and sugars without a participating group on C-2\textsuperscript{135} a nearly constant product ratio of α/β=1 was obtained which was not influenced by variation of the reaction conditions\textsuperscript{141}.

The reactions with sugars bearing a 2α-acyloxy group show a high degree of stereoselectivity, with respect to the sugar, in that only β-glycosyl derivatives are obtained. (Some production of α-anomers has been observed\textsuperscript{142} when very small amounts of SnCl\textsubscript{4} are used.) With respect to
\[ \text{S}-\text{OAc} \]

- 110 -

\[ \text{SnCl}_4 \]
\[ \text{ClCH}_2\text{CH}_2\text{Cl} \]
or \[ \text{CH}_3\text{CN} \]

- 111 -

- 112 -

- 113 -

\[ \text{Me}_3\text{SiO}^\text{N}^\text{R}_1 \]

\[ \text{N}^\text{R}_2 \]

- 109 -

\[ \text{a) } R_1 = H = R_2 \]
\[ \text{b) } R_1 = H; R_2 = \text{NO}_2 \]
\[ \text{c) } R_1 = H; R_2 = \text{OMe} \]
\[ \text{d) } R_1 = H; R_2 = \text{N}^\text{R}_2 \]
\[ \text{e) } R_1 = \text{CH}_3; R_2 = \text{CH} \left( \text{CH}_3 \right)_2 \]
\[ \text{f) } R_1 = \text{CH}_3; R_2 = \text{CH} \left( \text{CH}_3 \right)_2 \]
\[ \text{g) } R_1 = \text{CH}_3; R_2 = \text{NO}_2 \]
the base, a complex combination of electronic and steric factors determine the rate of the reaction and the type of product obtained. Thus, depending on the pyrimidine derivative used, one may obtain N-1 nucleosides (111), N-3 nucleosides (112), or N-1, N-3-bis-nucleosides (113). The study of Niedballa and Vorbrüggen 140 on the influence of 5 and 6 substituents in silylated uracils (109) is an illustrative example. The results of the reactions are summarized in Table I. The findings seem to support the contention of the authors that the SnCl₄-catalyzed silyl Hilbert-Johnson reaction between the hard 143 sugar cation and the hard 143 nitrogen of the silylated base is similar to Friedel-Crafts acylations and that the formation of stable donor-acceptor complexes between the catalyst and base prevent or impede the acylation by hard 143 acyl cations. Thus, electron-donating substituents on C-5 of the base such as OCH₃ (109c) or NR₂ (109d), result in formation of stable 1:1 donor-acceptor or π complexes analogous to complex 108 so that these bases only react with compound 110 if additional SnCl₄ is added to effect the formation of sugar cations. Even with an excess of catalyst the reaction rates of 109c and 109d are much slower than that of 5-nitrouracil (109b) where no complex formation seems to occur. Such complex formation also seems to favour the formation of the apparently kinetically and (in the presence of a 6 substituent) sterically favoured N-3 product 112
TABLE 1. Reaction of 1-0-Acetyl-2,5-tri-0-benzoyl-β-D-ribofuranose (110) with Silylated Uracils (109) in the Presence of SnCl₄ at 23°C.

<table>
<thead>
<tr>
<th>Uracil (silylated)</th>
<th>SnCl₄ equiv</th>
<th>Time, h</th>
<th>N₁ %</th>
<th>N₃ %</th>
<th>N₁,N₃ %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>a</td>
<td>b</td>
<td>a</td>
</tr>
<tr>
<td>Uracil</td>
<td>.7</td>
<td>.7</td>
<td>12</td>
<td>12</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>1.4</td>
<td>1</td>
<td>73</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>6-Azauracil</td>
<td>.2</td>
<td>.2</td>
<td>4</td>
<td>12</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>1.4</td>
<td>1</td>
<td>90</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>5-Nitouracil</td>
<td>.2</td>
<td>.2</td>
<td>0.1</td>
<td>0.1</td>
<td>97</td>
</tr>
<tr>
<td>5-Methoxyuracil</td>
<td>1.4</td>
<td>.9</td>
<td>25</td>
<td>12</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>1.4</td>
<td>2</td>
<td>1</td>
<td></td>
<td>39</td>
</tr>
<tr>
<td>5-Morpholinouracil</td>
<td>12</td>
<td>12</td>
<td>72</td>
<td>24</td>
<td>18</td>
</tr>
<tr>
<td>5,6-Dimethyluracil</td>
<td>1.1</td>
<td>1.1</td>
<td>6</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>6-Methyl-5-nitouracil</td>
<td>9</td>
<td>9</td>
<td>09</td>
<td>06</td>
<td>84</td>
</tr>
</tbody>
</table>

a In 1,2-dichloroethane. b In acetonitrile.

Data from references 136 and 140.

and the N-1, N-3 product 113. Conversely, when complex formation is inhibited electronically (109b and g), sterically (compare 109f and 109e), or by the use of a more polar solvent (acetonitrile) then in most cases the N-1/ N-3 product ratio is increased.
In 1974, Lichtenthaler et al.\textsuperscript{146} used \( \text{SnCl}_4 \) to catalyze the synthesis of a number of \( \text{N}^6 \)-benzoyl-adenine \( \beta \)-nucleosides from per-acetylated sugars and \( \text{N}^6 \)-benzoyl-\( \text{N}^6,9 \)-bis(trimethylsilyl)adenine (114).

\[
\text{OAcyl} + \begin{array}{c}
\text{Me}_3\text{SiNBz} \\
\text{SiMe}_3
\end{array}
\xrightarrow{\text{SnCl}_4, \text{C}_2\text{H}_4\text{Cl}_2, 60-70^\circ} \begin{array}{c}
\text{HN}_{\text{Bz}} \\
\text{OAcyl}
\end{array}
\]

The reaction offers certain advantages over the methods previously discussed. Firstly, the method is applicable to a wide range of purine derivatives. In the non-catalytic fusion\textsuperscript{117,113} method (see section 4.2.1) only "acidic" purines, such as 6-chloro, 2,6-dichloro, 2,6,8-trichloro, and 6-cyano-purine, gave nucleoside products, whereas 6-methoxypurine (115; \( R_1 = \text{Me}, R_2 = \text{H} \)) did not. The use of the \( \text{SnCl}_4 \) method with the corresponding silyl derivative (115; \( R_1 = R_2 = \text{SiMe}_3 \)) and per-acyl sugar 110 afforded\textsuperscript{146} tribenzoylinosine (116) in a 71\% yield.

Secondly, this method shows a high degree of stereoselectivity favouring the formation of \( \beta \)-nucleosides. In catalyzed fusion reactions\textsuperscript{118} and \( \text{TiCl}_4 \) catalyzed con-
densations of chloromercuri-purines\textsuperscript{147-149} the product is often a mixture of α,β anomers. The stereochemistry of the reaction is probably controlled by the 2-α-acyloxy group via a mechanism analogous to the auto-catalyzed fusion reaction (section 4.2.1) and the mercury salt catalyzed Hilbert-Johnson procedure (section 4.2.3.1).

4.2.3.3 Perfluoroalkane Sulfonate and Perchlorate Catalysts

In 1975, Vorbrüggen and Krolikiewicz reported\textsuperscript{150} the synthesis of a number of β-nucleosides by reaction of 1,2-dichloroethane solutions of per-acyl ribofuranose \textsuperscript{110} with silylated pyrimidines and purines in the presence of trimethylsilyl perchlorate or trimethylsilyl trifluoromethane sulfonate. The most important feature of these reactions, as compared to the analogous SnCl\textsubscript{4} catalyzed reactions, is the striking absence of N-3 and N-1, N-3 nucleoside by-products\textsuperscript{136,140,151}. Similar success has been achieved\textsuperscript{152,153} using 2,3,5-tri-0-benzoyl-\textbeta-ribo-
furanosyl bromide and silver trifluoromethyl sulfonate (silver triflate).

Very recently, Vorbrüggen and Bennua have reported a procedure for the in situ generation of both the silylated heterocycle and catalyst to effect nucleoside formation in one step. As an example of the procedure, uracil was reacted with 1.0 equivalent of acyl sugar 110 and 2.4 equivalents of potassium nonaflate in absolute acetonitrile in the presence of 3.1 equivalents of trimethylchlorosilane (TCS) and 0.7 equivalents of hexamethyldisilazane (HMDS) to afford N-1-(2',3',5'-tri-O-benzoyl-β-D-ribofuranosyl) uridine (111a) in 84% yield. Comparable results were obtained in analogous reactions using free trifluoromethane sulfonic acid or SnCl₄ as catalyst; (81% and 83% yields,
respectively, were achieved). This compares very favourably with the results shown in Table I.

4.3 Peptidyl Nucleosides

Besides the interest that has been shown in branched-chain sugars in general, there has been particular interest shown in amino sugars. Amino sugars occur as the basic constituents of many antibiotics and many of these nucleoside antibiotics contain amino acid functionalities and/or peptide linkages attached to the sugar. Blasticidin S (117), gougerotin (118), and puromycin (119) are examples of such peptidyl nucleosides.

\[
\begin{align*}
\text{117} & \quad \text{118} & \quad \text{119}
\end{align*}
\]
A new group of peptidyl nucleosides that are antifungal in their action is the polyoxins.

4.3.1 The Polyoxins

The result of a screening program for antibiotics active against a photopathogenic fungus, *Pellicularia filamentosa* f. *sasakii*, which causes sheathblight in rice plants, was the recovery of a polyoxin complex from the culture broths of three strains of *Streptomyces* belonging to *S. cacaoi*. To date thirteen polyoxins (designated A to M) have been isolated and characterized.

The structures of the polyoxins, as elucidated by Isono *et al.* 161-164, are depicted in Table 2. Each of the polyoxins contains one of four possible (S)-amino uronucleosides - each nucleoside differing only in the nature of its pyrimidine base. Each of the polyoxins, except polyoxin C, contains one or two of three possible L-amino acids which are designated polyoximic acid (121), carbamoylpolyoxamic acid (122) and carbamoyldeoxypolyoxamic acid (123).

The polyoxins show highly specific activity against photopathogenic fungi 156,158,165,166 while exhibiting no toxicity towards bacteria, food crops, fish, or laboratory animals 156,158. A recent study has also shown 167 that polyoxin A is a more effective inhibitor of tobacco mosaic virus than blasticidin S.
TABLE 2. Structure of the Polyoxins (120).

<table>
<thead>
<tr>
<th>Polyoxin</th>
<th>$R_1$</th>
<th>$R_2$</th>
<th>$R_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>CH$_2$OH</td>
<td>COOH</td>
<td>OH</td>
</tr>
<tr>
<td>B</td>
<td>CH$_2$OH</td>
<td>OH</td>
<td>OH</td>
</tr>
<tr>
<td>C</td>
<td>COOH</td>
<td>OH</td>
<td>OH</td>
</tr>
<tr>
<td>D</td>
<td>COOH</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>E</td>
<td>COOH</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>F</td>
<td>COOH</td>
<td>COOH</td>
<td>OH</td>
</tr>
<tr>
<td>G</td>
<td>CH$_2$OH</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>H</td>
<td>CH$_3$</td>
<td>COOH</td>
<td>OH</td>
</tr>
<tr>
<td>J</td>
<td>CH$_3$</td>
<td>OH</td>
<td>OH</td>
</tr>
<tr>
<td>K</td>
<td>H</td>
<td>COOH</td>
<td>OH</td>
</tr>
<tr>
<td>L</td>
<td>H</td>
<td>OH</td>
<td>OH</td>
</tr>
<tr>
<td>M</td>
<td>H</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>R</td>
<td>OH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td></td>
<td>COOH</td>
<td></td>
</tr>
</tbody>
</table>
The first indications of the mode of action of the polyoxins came with the observation that cells grown in the presence of inhibitory concentrations of the complex were osmotically fragile and prone to bursting\textsuperscript{166,168-170}. This was taken to be indicative of weakened cell walls and investigations were then focused upon the effects of polyoxins on cell wall synthesis\textsuperscript{171}. It was subsequently determined that the polyoxins impede cell wall formation by inhibiting the enzyme chitin synthetase which is responsible for the polymerization of N-acetyl glucosamine to afford chitin.

After the publication of the structural elucidation of the polyoxin complex, it was not until 1971 that the first synthesis of a polyoxin component was reported. Naka\textsuperscript{172} and co-workers were the first with their synthesis of methyl(5-benzamido-3-\textsubscript{O}-benzoyl-5-deoxy-1,2-\textsubscript{O}-isopropylidene-\textalpha--;\textupsilon--allofuran)uronate (124). A few months later Moffatt and co-workers reported\textsuperscript{173} the synthesis of uracil.
polyoxin C \((125a)\) and its \(\alpha-L\)-talo-isomer \((125b)\). The first synthesis of an existing polyoxin was carried out by Kuzuhara and Emoto. Their synthesis of the most active \(^{171}\) of the polyoxins, polyoxin J. \((120-J)\), was composed of three parts:

1) synthesis of thymine polyoxin C \((126)\)^{174},
2) synthesis of carbamoylpolyoxamic acid (122)\textsuperscript{175} and 3) peptidyl coupling of the above two components\textsuperscript{176}.

5. Synthesis of Glycosyl* Amino Acids

Many of the syntheses of glycosyl amino acids reported to date have incorporated reactions involving an $S_N^2$ displacement of a secondary methanesulfonyloxy or toluenesulfonyloxy group with sodium azide followed by reduction of the resulting azide to afford an amine.

The methods of Naka\textsuperscript{172} and Emoto\textsuperscript{174} both employed two successive displacement reactions in order to achieve the correct stereochemistry in the final product. The readily available fully blocked sugar (127) was treated with sodium benzoate to afford the di-O-benzoyl compound 128 with inversion of configuration at C-5. De-O-benzylation of compound 128, followed by selective tritylation of the C-6 hydroxyl and sulfonylation of the C-5 hydroxyl, afforded compound 129. Treatment of compound 129 with sodium azide gave the azido sugar 130 which was then reduced to give an amino sugar with the correct configuration at C-5.

In Moffatt's\textsuperscript{173} synthesis of uracil polyoxin C (125a) the aldehydo-nucleoside 131 was treated with potassium carbonate and sodium cyanide to give epimeric cyanohydrins which were immediately reacted with hydrogen peroxide to

* used in an extended sense.
give a mixture of epimeric hydroxyamides from which the \( \alpha-L\)-taluronamide \( 132 \) was separated. The \( \text{C}-5 \) hydroxy of compound \( 132 \) was then mesylated and the resulting \( \text{O}-\)mesyl group displaced by azide ion to give the \( 5\)-azido-\( \beta-D\)-alluronamide \( 134 \). Hydrolysis of the amide and cyclohexylidene groups and reduction of the azide functionality of compound \( 134 \) thus afforded the \( D\)-uronic acid \( 125a \).

Rosenthal, Shudo, and Richards\(^{177-179}\) have prepared a number of analogues of the sugar portion of the polyoxins in which the \( \alpha\)-amino acid is attached to \( \text{C}-3 \) of the sugar instead of \( \text{C}-4 \). The initial compounds in the reaction
sequences were the E and Z unsaturated esters (135 and 136, respectively) previously prepared by Rosenthal and Nguyen.83

Stereospecific hydroxylation177 of compound 136 with potassium permanganate followed by acetylation of the secondary hydroxyl group yielded the (S)-α-O-acetyl ester 137. Stereoselective dehydration of the tertiary alcohol
with thionyl chloride yielded the enol acetate 138 which in turn was hydrogenated to afford 3-C-[(R)-acetoxy-(methoxycarbonyl)methyl]-3-deoxy-1,2:5,6-di-O-isopropylidene-\(\alpha\)-D-allofuranose (139, R=Ac). The acetyl blocking group was then hydrolyzed and replaced with a tosyl group. When compound 139 (R=Ts) was treated with sodium azide it underwent the expected displacement reaction, with inversion of configuration, to yield the azide 140 which was immediately reduced to give the \(L\)-amino ester 141. Use of the \(E\)-unsaturated ester 135 in an analogous sequence of reactions yielded the corresponding \(D\)-amino ester 142 and by omitting
the dehydration reaction from the sequence the C-3-hydroxyl amino acids 143 and 144 were prepared\textsuperscript{178}.

Jordaan and co-workers\textsuperscript{180-182} have employed a different method for the synthesis of glycosyl amino acids. Condensation of ethyl isocyanoacetate and the keto-sugar using sodium hydride in tetrahydrofuran afforded the unsaturated formylamino ester 145 as the major product. The saturated D-formyl amino ester 146 was obtained by reduction of compound 145 with Raney nickel.

Compound 146 was degraded to a pentofuranose in the usual manner and was characterized as its crystalline p-bromobenzenesulphonate 147. X-ray analysis\textsuperscript{183} of compound 147 established that the branch-chain possessed (R) stereochemistry. An unambiguous proof of structure of the \(\alpha\)-amino esters synthesized by Rosenthal \textit{et al.}\textsuperscript{177-179}. 

\[
\begin{align*}
\text{141} & \quad R_1=H; R_2=\text{NH}_2 \\
\text{142} & \quad R_1=\text{NH}_2; R_2=H \\
\text{143} & \quad R_1=H; R_2=\text{NH}_2 \\
\text{144} & \quad R_1=\text{NH}_2; R_2=H
\end{align*}
\]
was provided\textsuperscript{179} by conversion of the D-amino ester \textsuperscript{142} into the N-salicylidene compound \textsuperscript{148} which was identical to a compound synthesized from the D-formylamino ester \textsuperscript{146}\textsuperscript{180}.

When the condensation of ethyl isocyanoacetate and ketose \textsuperscript{25} was performed in ethanol, using sodium cyanide as the catalyst, the D-formylamino ester allose derivative \textsuperscript{149} was the product\textsuperscript{180}. The attempted degradation of compound \textsuperscript{149} into its pentofuranose derivative led to the formation of the aminal \textsuperscript{150}\textsuperscript{181}. Acetolysis of compound
caused acetal migration to yield compound 152 which was condensed with bis(trimethylsilyl)uracil to afford the β-nucleoside 153. The 2,3-0-isopropylidene group of 153 could not be hydrolyzed without effecting cleavage of the N-glycosyl linkage. Although the tetra-acetate 154 was subsequently prepared by acetolysis of the di-acetate 151, all attempts to synthesize a nucleoside using 154 failed.
Jordaan and co-workers\textsuperscript{182} have recently synthesized the C-2 glycosyl N-formylamino esters 155 and 156 using a similar approach.

\[
\begin{align*}
155 & \quad \text{CO}_2\text{Et} \\
\text{OHCH}_2\text{N} - & \quad \text{H} \\
\text{O} & \quad \text{OMe}
\end{align*}
\]

\[
\begin{align*}
156 & \quad \text{CO}_2\text{Et} \\
\text{H} & \quad \text{NHCHO} \\
\text{O} & \quad \text{OMe}
\end{align*}
\]

Rosenthal and Dooley\textsuperscript{184} have applied the azlactone synthesis\textsuperscript{185} to the keto-sugar 25 to afford the azlactones 157 and 158 which, after methanolysis and reduction, yielded the benzamido derivatives of compounds 141 and 142.

\[
\begin{align*}
157 & \quad \text{Ph} \\
\text{X} & \quad \text{O} \\
\text{N} & \quad \text{O} \\
\text{O} & \quad \text{O}
\end{align*}
\]

\[
\begin{align*}
158 & \quad \text{Ph} \\
\text{X} & \quad \text{O} \\
\text{N} & \quad \text{O} \\
\text{O} & \quad \text{O}
\end{align*}
\]
Umezawa and co-workers\textsuperscript{186} used the Bucherer hydantoin synthesis\textsuperscript{187} to prepare the hydantoin\textsuperscript{159} which was hydrolysed to afford the partially blocked cyclic $\alpha$-amino acid\textsuperscript{160}. The amino acid\textsuperscript{160} was subsequently used to synthesize the glycosyl amino acid nucleoside\textsuperscript{161}.

\begin{itemize}
  \item \textbf{159}
  \item \textbf{160}
  \item \textbf{161}
\end{itemize}
III. RESULTS AND DISCUSSION

1. Synthesis of Branched-chain Amino Sugars. Reaction of 1,2:5,6-Di-O-isopropylidene-α-D-ribo-hexofuranos-3-ulose with Sodium Cyanide and Methyl Nitroacetate.

Although the Kiliani and Fischer cyanohydrin synthesis\textsuperscript{61} has been widely applied to aldoses the procedure has been very little used to prepare branched-chain sugars from ketoses. Tronchet\textsuperscript{62} and Bourgeois\textsuperscript{63,64} have recently reported application of the cyanohydrin synthesis to 1,2:5,6-di-O-isopropylidene-α-D-ribo-hexofuranos-3-ulose (25) to yield an epimeric mixture of cyanohydrins but the structures of the cyanohydrins were not proven.

In this thesis the proof of structure of the cyanohydrins derived from ketose 25 and the effect of the pH on controlling the proportions of products will be described. In addition, some of the preliminary work dealing with the branched-chain methyl nitroacetate adduct of the same ketone, which will be dealt with in detail in Sections 3.3 and 3.4, will be presented.
1.1 Synthesis of 3-C-Cyano-1,2:5,6-di-0-isopropylidene-\(\alpha\)-D-glucofuranose (26) and 3-C-Cyano-1,2:5,6-di-0-isopropylidene-\(\alpha\)-D-allofuranose (27).

The reaction of sodium cyanide, methyl nitroacetate, and ketose 25 was found to produce either the gluco-cyano-hydrin 26 or the allo-cyanohydrin 27 depending upon the order of addition of the reactants. The results are summarized in Table 3.

1.1.1 Procedure A to Yield 3-C-Cyano-1,2:5,6-di-0-isopropylidene-\(\alpha\)-D-glucofuranose (26).

The addition of an equivalent of sodium cyanide to a solution of ketose 25 caused the solution to turn black immediately. After the reaction mixture was stirred at room temperature for 18 hours, the reaction was worked up and the crude product was chromatographed on a column of silica gel, using 1:1 benzene-ethyl acetate as the developer, to afford unreacted ketose 25 (31%) and a clear colourless syrup. The gluco-cyanohydrin 26 crystallized spontaneously upon standing.

The p.m.r. spectrum of the syrup remaining after the removal of the crystalline gluco-cyanohydrin 26 showed two doublets at \(\delta 4.55\) and \(\delta 4.92\) which were attributed to the C-2 protons of the gluco and allo cyanohydrins, respectively. Based on the relative intensities of these resonances, the weight of the mother liquor, and the weight of crystalline
compound 26, it was calculated that the reaction yielded the \textit{gluco}-cyanohydrin 26 in 35\% yield and the \textit{allo}-cyanohydrin 27 in 3\% yield (based on ketose consumed).

The yield of compound 26 was increased to 57\% by adding, after the addition of sodium cyanide to the ketose 25, one molar equivalent of methyl nitroacetate. The yield of the accompanying compound 27 was calculated to be 4\% in this case.

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline
\textbf{PROCEDURE} & \textbf{NaCN (equiv)} & \textbf{MNA (equiv)} & \textbf{SOLVENT (ml)} & \textbf{REACTION TIME (h)} & \textbf{TEMP (°C)} & \textbf{CYANOHYDRIN} & \textbf{CMPD 162 (\%)} \\
\hline
A & 1 & none & EtOH(5) & 18 & 20 & 35 & 3 & - \\
A & 1 & 1 & EtOH(2) & 18 & 20 & 57 & 4 & - \\
B & 1 & 1 & EtOH(5) & 18 & 22 & 6 & 85 & 7 \\
B & 1 & none & EtOH(5) & 18 & 22 & 5 & 71 & - \\
\hline
\end{tabular}
\caption{Application of the Cyanohydrin Synthesis to Ketose 25 in the Presence of Methyl Nitroacetate (MNA).}
\end{table}
1.1.2 Procedure B to Yield 3-C-Cyano-1,2:5,6-di-0-isopropylidene-α-D-allofuranose (27).

In this procedure it was found that when the order of addition of methyl nitroacetate to ketose 25 was changed from that of procedure A, then the allo-cyanohydrin 27 was formed predominantly. Thus, addition of ketose 25 to a previously formed equimolar mixture of sodium cyanide and methyl nitroacetate in ethanol gave, after chromatography on silica gel using 4:6 benzene-ethyl acetate as developer, unreacted ketose (22%) and a clear colourless syrup. The allo-cyanohydrin 27 was crystallized from the syrup using benzene-hexane and an analysis of the p.m.r. spectrum of the mother liquor revealed the presence of resonances attributable to H-2 of both cyanohydrin epimers as well as a sharp singlet at δ3.83 attributed to the methyl ester of 1,2:5,6-di-0-isopropylidene-3-C-{(R,S)-nitro(methoxy carbonyl)methyl} -α-D-allofuranose (162). (Compound 162 and its proof of structure will be dealt with in detail in Sections 3.3 and 3.4.) As was done for procedure A, it was calculated that procedure B gave the allo-cyanohydrin 27, the gluco-cyanohydrin 26, and compound 162 in yields of 85%, 6% and 7%, respectively, based on consumed ketose. Attempts to obtain pure compound 162 by repeated chromatography were unsuccessful because 162 was quite unstable.
1.1.3 Procedure C to Yield 3-O-Acetyl-3-C-cyano-1,2:5,6-di-O-isopropylidene-α-D-gluco and allofuranose (163) and (164), 3-O-Acetyl-1,2:5,6-di-O-isopropylidene-3-C-[(R,S)-nitro(methoxycarbonyl)methyl]-α-D-allofuranose (165) and 3,5,6-tri-O-Acetyl-3-C-cyano-1,2-O-isopropylidene-α-D-allofuranose (166).

Because of the instability of compound 162 towards column chromatography, it was decided to repeat procedure B and to derivatize the products prior to chromatography in order to determine whether or not compound 162 was produced in greater than 7% yield.

When a molar equivalent of ketose 25 was added to a mixture of one equivalent of sodium cyanide and one equivalent of methyl nitroacetate in ethanol and the resulting mixture allowed to react for 18 hours at room temperature, followed by an immediate acetylation of the partially purified product mixture with acetic anhydride in the presence of p-toluenesulfonic acid monohydrate, four products were obtained. The mixture of products was chromatographed on silica gel, using 9:1 benzene-ethyl acetate as the developer, to afford the 3-O-acetyl derivatives of each product, namely, the gluco-cyanohydrin 163, the allo-cyanohydrin 164, the methyl nitroacetate adduct 165, and the tri-O-acetyl allo-cyanohydrin 166 in a ratio of 1:23:4:72, respectively.
A proof of structure of compounds 163 and 164 was provided by acid catalyzed acetylation of the pure cyano-hydrins 26 and 27 to afford compounds identical (m.p., n.m.r., optical rotation) to those obtained by procedure C. Proof of structure of compound 166 was provided by selectively hydrolyzing the 5,6-O-isopropylidene group of the allo-cyano-hydrin 164 and subsequently acetyling the product to afford a substance identical in all respects with the tri-O-acetyl allo-cyanohydrin 166. This selective acetolysis of the 5,6-O-isopropylidene group of a di-O-isopropylidene sugar derivative using p-toluenesulfonic acid monohydrate is noteworthy as it can provide a means of markedly reducing the number of steps required in some nucleoside syntheses.

1.2 Synthesis of 3-C-Acetamidomethyl-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (54) and 3-C-Acetamidomethyl-1,2:5,6-di-O-isopropylidene-α-D-allofuranose (53).

The proof of structure of the epimeric cyanohydrins was provided in the following way. Lithium aluminum hydride reduction of the gluco-cyanohydrin 26 followed by acetylation of the amino product, afforded the corresponding 3-C-acetamidomethyl gluco-derivative 54. Compound 54 has a p.m.r. spectrum identical to that of a substance which was obtained by application of the nitromethane synthesis to ketose 25 followed by reduction and acetylation. Unequivocal proof of structure of that compound was provided by Yoshimura et
Similarly, reduction and acetylation of the allo-cyanohydrin 27 gave the known\textsuperscript{98} 3-\textsubscript{\textalpha{}}-acetamidomethyl allo-sugar 53.

In 1973, Rosenthal and Baker reported the synthesis of a number of analogues of the amino sugar nucleoside moiety of puromycin (119). Their synthetic sequence utilized the previously prepared 3-C-cyanomethyl-3-deoxy hexofuranoside 36 which was degraded by the usual series of reactions to afford 5-0-benzoyl-3-C-cyanomethyl-3-deoxy-1,2-0-isopropylidene-α-D-ribofuranose (167). When compound 167 was treated with 90% trifluoroacetic acid in order to hydrolyze the acetal blocking group, and the resulting product mixture was acetylated, not only was the expected 1,2-di-0-acetyl-3-C-cyanomethyl sugar 168 isolated but also a small amount (~5%) of the 2,3-γ-lactone 169. Obviously, the acid hydrolysis of the 1,2-0-isopropylidene group was accompanied by partial hydrolysis of the nitrile group giving a carboxylic acid which then underwent intramolecular esterification to yield a 2,3-γ-lactone.

In any nucleoside synthesis reaction, compound 169 would be unable to form the 1,2-acyloxonium ion intermediate which leads to predominant formation of β-nucleosides.
(see the Introduction, section 4.2). It seemed to us, therefore, that a quantitative synthesis of lactone \textit{169} would provide a facile route to the less commonly synthesized α-nucleosides. We thus set out to prepare compound \textit{169} for use in the synthesis of α and β-adenine nucleoside analogues of the antibiotic puromycin.

2.1 \textbf{Synthesis of 1-0-Acetyl-5-0-benzoyl-3-C-carboxymethyl-3-deoxy-β-D-ribofuranose-2,3-γ-lactone (169).}

Rather than trying to hydrolyze the cyanomethyl group of compound \textit{167}, it seemed advantageous to introduce the
carboxylic acid functionality at the beginning of the synthetic sequence.

Using a previously reported procedure, the ylid generated by the reaction of potassium tert-butoxide and carbomethoxymethylidimethyl phosphonate was condensed with the keto-sugar and the resulting mixture of isomeric α,β-unsaturated esters (135 and 136) was hydrogenated over palladium-on-charcoal to afford the known 3-C-(carbo-

\[
\begin{align*}
25 & \rightarrow \text{ MeO}_2\text{C} \quad \text{135} \\
& \quad \text{H} \\
& \quad \text{CO}_2\text{Me}
\end{align*}
\]

\[
\begin{align*}
& \text{RO} \\
171 & \quad \text{R} = \text{H} \\
172 & \quad \text{R} = (\text{C}_6\text{H}_5)\text{CO}
\end{align*}
\]
methoxymethyl)-3-deoxy-1,2:5,6-di-O-isopropylidene-α-D-allofuranose (35). The 5,6-O-isopropylidene blocking group of compound 35 was selectively hydrolyzed by dissolving the sugar in 66% aqueous acetic acid. The reaction was carefully monitored by t.l.c., using 4:6 benzene-ethyl acetate as developer, and when the starting material (R_f 0.6) could no longer be detected, or when compounds having a lower R_f than the desired product (R_f 0.2) were detected, the reaction was stopped by evaporating the acetic acid and water under reduced pressure. The resulting product mixture crystallized spontaneously to afford 3-C-(carbomethoxymethyl)-1,2-O-isopropylidene-α-D-allofuranose (170) quantitatively and of such purity that it could be used for subsequent reactions without further purification. Compound 170 was degraded to 3-C-(carbomethoxymethyl)-3-deoxy-1,2-O-isopropylidene-α-D-ribofuranose (171) by treatment with sodium periodate followed by immediate reduction with sodium borohydride. Benzoylation of compound 171 yielded 5-O-benzoyl-3-C-(carbomethoxymethyl)-3-deoxy-1,2-O-isopropylidene-α-D-ribofuranose (172).

The 1,2-O-isopropylidene group of compound 172 was hydrolyzed using a 90% (v/v) trifluoroacetic acid solution. After the reaction mixture was stirred for 1 1/2 hours at room temperature, the volatile components were evaporated in vacuo to yield a white solid which was presumed to be the 2,3-γ-lactone 173. This compound was not
characterized but was immediately acetylated to afford a compound which was identical in all respects (m.p., optical rotation, n.m.r.) to the γ-lactone 169 obtained by Rosenthal and Baker. The synthetic sequence yielded compound 169 from compound 35 in an overall yield of 66%.

2.2 Synthesis of 2,6-Dichloro-9-[3'-C-(carboxymethyl-2',3'-γ-lactone)-3'-deoxy-β and α-D-ribofuranosyl]purine (174 and 175).

The initial nucleoside synthesis attempted with the lactone sugar 169 was a fusion reaction with 2,6-dichloro-purine. The sugar and one equivalent of the base were intimately mixed, dried, and then fused at 160° and 20 torr. After approximately 5 minutes, the initial vigorous bubbling of the melt had subsided and the pressure was reduced to
0.1 torr for a further 15 minutes. After cooling, the amber glass was chromatographed to afford unreacted starting material 169 (38%), β-nucleoside 174 (23%), and α-nucleoside 175 (27%).

In an attempt to reduce the amount of unreacted starting material and increase the yields of the products, a number of small scale reactions (~1 mg. of materials) was carried out in which the lactone/base ratio, reaction time, and temperature were varied. The results of these tests were judged qualitatively by t.l.c. and the more promising results repeated on a larger scale (30-50 mg. of lactone) to allow a quantitative analysis of the products obtained. Some of the conclusions arrived at include:-

a) a base/lactone ratio >1 favoured consumption of the starting material but when the ratio approached a value of 2 or greater then practical difficulties were encountered in separating the products from the unreacted 2,6-dichloropurine.

b) at temperatures much higher than 160° decomposition of the base was observed along with sublimation of the lactone 169.

c) longer reaction times led to increased yields of nucleosides.

The optimum conditions found for this system involved fusing a 1.5 to 1 ratio of 2,6-dichloropurine and lactone 169 at 160° and 20 torr for 5 minutes, then at 140° and
0.2 torr for an additional 25 minutes. Under these conditions the amount of unreacted lactone 169 was reduced to 8% while the yields of the α and β-nucleosides, (175, 174) were 41% and 48%, respectively. The addition of acid catalysts (dichloroacetic acid or p-toluenesulphonic acid) to the fusion reaction did not produce any discernable change in the results.

\[ \text{169} \rightarrow \text{174} + \text{175} \]

The anomeric configurations of the nucleosides were assigned on the basis of their p.m.r. spectra. The anomeric proton of the β-nucleoside was observed as a singlet at \( \delta 6.24 \). However, the H-2' proton appeared as a quartet at \( \delta 5.64 \) with coupling constants of 1.0 Hz and 6.8 Hz which suggested that the H-1' resonance was in fact an unresolved doublet with \( J_{1',2'} = 1.0 \) Hz. The anomeric proton of the α-nucleoside appeared as a doublet at \( \delta 6.80 \) coupled to the
H-2' proton at δ5.46 with a J value of 4.5 Hz and the H-2' proton was coupled to H-3' with J_{2',3'} equal to 7.0 Hz. The relative magnitudes of the H-1', H-2' coupling constants and the fact that the anomeric signal of the α-D-anomer is usually observed at lower field than that of the β-D-anomer confirm the assignments of the β-D-configuration to compound 174 and the α-D-configuration to compound 175.

2.3 Synthesis of 9-[3'-C-(Carboxymethyl-2',3'-γ-lactone)-3'-deoxy-β-D-ribofuranosyl]adenine (178) and 9-[3'-C-Carboxymethyl-3'-deoxy-α-D-ribofuranosyl]adenine (181).

The use of the reactive 2,6-dichloropurine in nucleoside syntheses has the disadvantage that subsequent reactions are necessary to convert the 2,6-dichloropurine nucleoside into its analogous adenine nucleoside. It was therefore deemed advantageous to attempt a condensation of lactone 169 directly with an adenine derivative. Towards this end, a dichloroethane solution of the lactone 169 and \( N^6 \)-benzoyl-\( N^6 \),9-bis(trimethylsilyl)adenine 191 was heated for 18 hours at 63° in the presence of stannous chloride. After work-up, the product was chromatographed on a column of silica gel to afford \( N^6 \)-benzoyl-9-(3'-C-carboxymethyl-2',3'-γ-lactone-3'-deoxy-β-D-ribofuranosyl)adenine 176 (31%), and the α-anomer 177 (31%).
The anomeric configuration of the nucleosides was made on the basis of their p.m.r. spectrum since the anomeric proton of the β-nucleoside 176 appeared as a singlet at δ6.20 while the anomeric proton of the α-nucleoside 177 appeared 0.69 p.p.m. further downfield as a doublet coupled to H-2' with $J_{1',2'} = 4.0$ Hz.
The benzoylated nucleosides were not characterized further but were immediately dried and dissolved in a methanol solution containing a catalytic amount of sodium methoxide. After a period of 7 days, Bio-Rex 70 (H⁺) cation exchange resin was added to remove the sodium ions. The filtrate remaining after the removal of the resin was evaporated in vacuo in order to remove the methyl benzoate by-product from the nucleoside products.

In the case of the methanolysis of the β-nucleoside 176, the lactone functionality was immediately regenerated by passing a methanol-water solution of the compound through a column of Bio-Rex 70 (H⁺) resin, using 8:2 methanol-water as the eluting solvent. The lactone β-nucleoside 178 crystallized spontaneously from the eluent and was removed by filtration in an overall 61% yield with respect to the blocked nucleoside 176. The u.v. spectrum of compound 178 exhibited maxima at 205 (ε18,700) and 257 nm (ε13,200) which confirmed the site of glycosylation at N-9\textsuperscript{190}.

In the 270 MHz p.m.r. spectrum of compound 178 (see Figure 1A), the existence of a lactone functionality was inferred by the presence of only one exchangeable hydroxyl proton (δ5.21) and no evidence of a low field acid proton resonance. This was confirmed by a carbonyl absorption at 1780 cm\textsuperscript{-1} in the i.r. spectrum of compound 178. The β-configuration of the nucleoside, suggested by the presence of a singlet anomeric proton resonance in the p.m.r. spectrum
Figure 1A  270 MHz P.M.R. Spectrum of 9-\{3'-C-(Carboxymethyl-2',3'-\gamma-lactone)-3'-deoxy-\beta-D-ribofuranosyl\}adenine (178) in DMSO-d$_6$. 
Figure 1B  Partial 270 MHz P.M.R. Spectrum of 9-[3'-C-(Carboxymethyl-2',3'-γ-lactone)-3'-deoxy-β-D-ribofuranosyl]adenine (178) in DMSO-d$_6$. 
(65.91), was confirmed by the c.d. spectrum which exhibited a negative Cotton effect\textsuperscript{220} at 260 nm.

The high resolution mass spectrum of compound 178 showed molecular ion and (M\textsuperscript{+} + 1) peaks at m/e values of 291.0953 and 292.1032, respectively, in excellent agreement with the corresponding theoretical values of 291.0967 and 292.1045. The elemental analysis of 178 also agreed with its proposed structure.

In the case of the methanolysis of the benzoylated lactone \(\alpha\)-nucleoside 177, a white crystalline product precipitated from solution after the neutralizing resin was removed by filtration. The elemental analysis of this product suggests that it was 9-(3'-C-carbomethoxymethyl-3'-deoxy-\(\alpha\)-D-ribofuranosyl)adenine (179).
In order to regenerate the lactone, a methanol-water solution of compound \textbf{179} was passed through a column of Bio-Rex 70 ($\text{H}^+$) resin using 8:2 methanol-water as the eluting solvent. Upon standing for 18 hours, a white crystalline product crystallized from the eluent and was removed by filtration in a 51\% yield, based on the blocked lactone $\alpha$-nucleoside \textbf{177}.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{reaction_diagram.png}
\end{figure}
The u.v. spectrum of the product exhibited maxima at 203 nm ($\varepsilon 17,900$) and 252 nm ($\varepsilon 12,700$) and established the product as an N-9 nucleoside. However, the 270 MHz p.m.r. spectrum of the product (see Figures 2A and 2B) showed it to be the 3'-C-carboxymethyl-$\alpha$-nucleoside rather than the expected lactone $\alpha$-nucleoside. The spectrum showed three exchangeable proton resonances in addition to the amine protons of the adenine base—a low field acid proton resonance at $\delta 12.13$ and two hydroxyl protons at $\delta 5.57$ and $\delta 4.83$. The presence of the acid functionality was confirmed by a broad absorption between 2400 and 3800 cm$^{-1}$ (OH) in the infra-red spectrum of the product. Also present in the p.m.r. spectrum was an anomeric proton resonance at $\delta 6.27$ with $J_{1',2'} = 3.0$ Hz. The lower field position and larger coupling constant of this resonance, compared to the analogous signal for the lactone $\gamma$-nucleoside ($\delta 5.91$, $J_{1',2'} = 0$ Hz), confirmed the $\alpha$-nucleoside confirmation of compound. Additional proof was provided by the c.d. spectrum of the compound, which exhibited a positive Cotton effect at 256 nm. The elemental analysis of the product was in agreement with its proposed structure.

The existence of the $\alpha$-nucleoside in the free acid form rather than the lactone form is most probably due to participation of the heterocyclic base in the hydrolysis of the lactone or by a favourable hydrogen-bonding
Figure 2A  270 MHz P.M.R. Spectrum of 9-[3'-C-Carboxymethyl-3'-deoxy-α-D-ribofuranosyl]-adenine (181) in DMSO-d₆.
Figure 2B  Partial 270 MHz P.M.R. Spectrum of 9-[3'-C-Carboxymethyl-3'-deoxy-α-D-ribo-furanosyl]adenine (181) in DMSO-d₆.
interaction between the base and the free acid which would favour the acid side of the acid-lactone equilibrium. A similar influence was shown by the heterocyclic base of 9-[3'-acetamido-3'-C-(carboxymethyl-2',3'-γ-lactone)-3'-deoxy-α-D-xylofuranosyl]adenine previously synthesized by Rosenthal and Ratcliffe\(^{222}\).

The mass spectrum of the carboxy α-nucleoside 181 is very similar to that of the lactone β-nucleoside 178 in that molecular ion and \((M^+ + 1)\) peaks appear at m/e values of 291.0984 and 292.1041, respectively. Obviously, under the operating conditions necessary to obtain a mass spectrum, compound 181 lost a molecule of water to yield the lactone α-nucleoside 180 and this was the compound whose mass spectrum was obtained.
3. **Synthesis of Analogue of the Nucleoside Moiety of the Polyoxins.**

The procedures employed, up until now, for the synthesis of glycosyl 3-C- amino acids have suffered from several disadvantages. The azlactone synthesis\(^\text{185}\) employed by Rosenthal and Dooley\(^\text{184}\) afforded 3-deoxy-N-benzoyl amino acids which could not be unblocked to yield the free amino acids. The stereospecific synthesis employed by Rosenthal et al.\(^\text{177-179}\) involves many steps and the overall yield is low. Finally, the condensation of ethyl isocyanoacetate with keto-sugars used by Jordaan and co-workers\(^\text{180-182}\) introduced a blocked amino acid possessing R-(D) stereochemistry whereas the naturally occurring polyoxins contain an S-(L)-amino acid. Furthermore, degradation of the resulting hexofuranose to afford a pentofuranose resulted in irreversible formation of an aminal\(^\text{181}\) which either would not undergo condensation with a pyrimidine base or could not be unblocked when condensation did occur.

In order to overcome the disadvantages encountered by these previous procedures, it was decided to attempt the condensation of methyl nitroacetate with various keto-sugars.

The primary objective was the synthesis of a peptidyl nucleoside composed of the following elements:

1) $\text{L-}(S)$-glycine linked through the $\alpha$-carbon to
2) C-3 of a D-allofuranose
and 3) a β-linked N-1 thymine base.

The synthetic strategy envisioned to accomplish this goal was as follows:
1) condensation of methyl nitroacetate with 1,2:5,6-di-O-isopropylidene-α-D-ribo-hexofuranos-3-ulose (25).
2) reduction of the nitro group and isolation of the desired allofuranos-3-yl L-amino acid.
and 3) condensation of the branch-chain sugar with a thymine base.

The work carried out towards this end is described in sections 3.2, 3.3, and 3.6.
At the same time investigations were made into:
1) synthesis of 3-deoxy allofuranos-3-yl branch-chain sugars (described in section 3.5)
and 2) synthesis of pentofuranos-3-yl branch-chain sugars (described in section 3.1).

3.1 Synthesis of 3-Deoxy-3-C-nitro(carbomethoxy)methylene- D-erythro-pentofuranoses.
In order to overcome the difficulties encountered by Jordaan and co-workers\textsuperscript{180-182} in their attempts to synthesize a pentofuranos-3-yl glycinate by degradation of a hexofuranos-3-yl glycinate, it was decided to attempt
the condensation of methyl nitroacetate directly with pentos-3-uloses.

3.1.1 Condensation of Methyl Nitroacetate with 5-O-Benzoyl-1,2-O-isopropylidene-\(\alpha\)-D-erythro-pentos-3-ulose (182).

The ketose 182 was obtained by a procedure which began with D-xylose. According to the procedure described by Baker and Schaub\(^{192}\), the di-0-isopropylidene compound 183, prepared by acid catalysed condensation of acetone with D-xylose, was treated with dilute acid to yield the monoacetone xylose 184. Compound 184 was then reacted with

\[\text{D-XYLOSE} \rightarrow 183 \rightarrow 185 \rightarrow 184\]
l.1 equivalents of benzoyl chloride to afford a product mixture from which 5-O-benzoyl-1,2-O-isopropylidene-α-D-xylofuranose (185) was separated by column chromatography in an 84% yield.

Two methods of oxidation of compound 185 were employed. In the first method, ruthenium tetroxide was used as an oxidant, according to a procedure reported by R.F. Nutt and co-workers\textsuperscript{193}. In our hands, however, the yields of the ketose 182 were too low to be useful due to concomitant formation of the product of oxygen insertion, namely, 6-O-benzoyl-3-deoxy-1,2-O-isopropylidene-3-oxa-α-D-erythro-hexos-4-ulose (186)\textsuperscript{193}.

\[
\begin{align*}
\text{BzO} & \quad \text{BzO} \\
\text{185} & \quad \text{182} \\
\text{186} & 
\end{align*}
\]

In the second method, the oxidation of compound 185 was accomplished using phosphorus pentoxide as the oxidant and acetic anhydride as the "activating" agent. Ketose 182 was thus obtained in >80% yield.

To a solution of the ketose 182 and ammonium acetate (1.1 equiv.) in anhydrous N,N-dimethylformamide
(DMF) was added methyl nitroacetate (2.1 equiv.). After the reaction was stirred for 2 hours, it was worked-up to yield a dark amber syrup. The t.l.c. of the crude product, using 8:2 benzene-ethyl acetate as the developer, showed

\[
\begin{align*}
\text{182} \xrightarrow{\text{NH}_4\text{OAc}, \text{MNA}, \text{DMF}} \\
\text{187} \quad R=H \\
\text{188} \quad R=\text{Ac}
\end{align*}
\]

the presence of two components with \( R_f \) values of 0.35 and 0.45. It was assumed that one of these products was the methyl nitroacetate adduct 187 and, therefore, the crude product mixture was dissolved in acetic anhydride and heated to \( \sim 90^\circ \) for 5 hours in the presence of a catalytic amount of p-toluenesulphonic acid monohydrate (p-TSA) in order to convert compound 187 into its more stable 3-O-acetyl derivative 188. Surprisingly, however, the reaction effected the total conversion of the lower \( R_f \) material into the higher \( R_f \) material together with substantial decomposition of the reactants. Column chromatography of the crude reaction mixture afforded (E) and (Z)-5-O-benzoyl-3-deoxy-1,2-0-isopropylidene-3-C-nitro(methoxycarbonyl)methylene-
α-D-erythro-pentofuranose (189) in an overall yield of 23% from compound 185.

The first indication of the structure of compound 189 appeared when the t.l.c. plates of the condensation and acetylation reactions were sprayed with a dilute solution of potassium permanganate. A positive result for unsaturation was exhibited by the higher \( R_f \) component. It is therefore suggested that the condensation reaction initially produced the adduct 187 which in turn underwent spontaneous dehydration to afford a mixture of compound 187 (\( R_f 0.35 \)) and the unsaturated nitro ester 189 (\( R_f 0.45 \)). The subsequent acid catalyzed acetylation conditions then drove the dehydration to completion to give compound 189 as the sole product.

The location of the carbon-carbon double bond in the branch-chain was evidenced by the shift to lower frequencies of the infra-red absorptions of the ester and nitro functionalities. The i.r. spectrum of compound 189 exhibits an ester absorption at 1730 cm\(^{-1}\) and a nitro absorption at 1545 cm\(^{-1}\), whereas the analogous absorptions for methyl nitroacetate occur at 1755 and 1570 cm\(^{-1}\).

The p.m.r. spectrum of compound 189 revealed a mixture of geometrical isomers. From the relative heights of methyl ester signals at 63.90 and 63.94, the ratio of isomers was calculated to be 1.9:1. The \( \mathrm{C}-2 \) proton of the major isomer, designated as H-2A, occurred at 65.52 as a
quartet with coupling constants of $J_{1,2} = 4.8$ Hz and $J_{2,4} = 1.8$ Hz while the C-2 proton of the minor isomer, designated as H-2B, occurred slightly downfield at $\delta 5.67$ with identical coupling constants. The magnitude of the $J_{2,4}$ values is consistent with values obtained for other systems involving a $\pi$-contribution to long-range couplings. In the unsaturated hexofuranoses $^{190-193}$ the magnitude of the H-2,

![Chemical Structures]

H-4 coupling was also indicative of the configuration at C-4 with the ribo sugars $^{190,179}$ and $^{191,177}$ possessing $J$ values of 1.5 Hz and 2.0 Hz, respectively, while the xylo sugars $^{192,196}$ and $^{193,196}$ had $J$ values equal to 0.0.

The elemental analysis of compound $^{189}$ was in agreement with its designated structure.

Because of the low yield of the product it was decided to attempt the condensation of methyl nitroacetate with the 5-O-benzyl ketose $^{194}$. 
3.1.2 Condensation of Methyl Nitroacetate with 5-0-Benzyl-1,2-0-isopropylidene-α-D-erythro-pentos-3-ulos (194).

Tosylation of monoacetone xylose 184 and subsequent treatment of the resulting 5-0-tosyl derivative 195 with an equivalent of base gave 1,2-0-isopropylidene-3,5-anhydro-α-D-xylofuranose (196)197. Compound 196 was then reacted with sodium benzylate to afford the 5-0-benzyl xylose 197198 which was oxidized199 using the ruthenium dioxide-sodium periodate method to afford the 5-0-benzyl ketose 194.
The condensation of methyl nitroacetate and ketose 194 was carried out as previously described for the 5-0-benzoyl ketose 182 and the product of the reaction was subjected to the same acid catalyzed acetylation conditions.

Chromatography of the resulting crude product mixture on a column of silica gel, which was packed and eluted with 9:1 benzene-ethyl acetate, afforded two components with \( R_f \) values of 0.50 and 0.28. The p.m.r. spectrum of the lower \( R_f \) material (58% by weight, based on ketose) showed that it was a mixture of compounds containing approximately 80% 5-benzyl ketose 194. The other fractions of this component were unidentified although the p.m.r. spectrum did not indicate the presence of a methyl ester nor did the i.r. spectrum show any absorptions attributable to a nitro group.

The higher \( R_f \) material (5% yield) possessed characteristics of an \( \alpha,\beta \)-unsaturated \( \alpha \)-nitro ester in that when a t.l.c. of this component was sprayed with potassium permanganate a positive test for unsaturation was obtained. The i.r. spectrum also exhibited ester and nitro absorptions at 1735 and 1545 cm\(^{-1}\), respectively. It is therefore proposed that this component is 5-0-benzyl-\( 3,\) deoxy-1,2-0-isopropylidene-\( 3-C \)-nitro(methoxycarbonyl)methylene-\( \alpha-D-\)erythro-pentofuranose (198).

In support of this proposal, the p.m.r. spectrum of compound 198 exhibited resonances for a total of 21 protons. Unlike the unsaturated \( \alpha \)-nitro ester 189, however, the
n.m.r. spectrum of compound 198 appeared to be of almost exclusively one geometrical isomer. The resonance of H-2 appeared at δ5.48 as a quartet with a J_{2,4} value of 1.5 Hz which, as previously discussed, is typical for compounds of such stereochemistry.

Unfortunately, a satisfactory analysis of compound 198 could not be obtained to help substantiate its proposed structure, and because of the extremely low yield of the product it was decided to abandon this approach in favour of condensations with the more readily available hexofuran-3-ulose 25.

3.2 Condensation of Methyl Nitroacetate with 1,2:5,6-Di-O-isopropylidene-α-D-ribo-hexofuranos-3-ulose (25).
3.2.1 Synthesis of 1,2:5,6-Di-0-isopropylidene-3-C-
[(R,S)-nitro(methoxycarbonyl)methyl]-α-D-
allofuranose (162).

Kornilov, Paidak and Zhdanov effected the condensation of ethyl nitroacetate with diisopropylidene-aldehyde-L-arabinose in an aqueous alcoholic medium using ammonium acetate as the condensing agent. However, the use of the ketose 25 in an aqueous medium results in the formation of an unreactive hydrate 199. In fact, the production of the ketose 25 involves ruthenium tetroxide oxidation 58,66 of the commercially available diisopropylidene glucose 200 to afford quantitatively the ketose hydrate 199 which must then be dehydrated in refluxing benzene or toluene.

Therefore, the first attempted condensations of ketose 25 and methyl nitroacetate in the presence of ammonium acetate employed anhydrous methanol, dimethylsulfoxide, or  N, N-dimethylformamide as solvents.
The most complete consumption of ketose, as evidenced by t.l.c., and the highest yields of products were obtained when two equivalents of methyl nitroacetate were added to a solution of ketose 25 and one equivalent of ammonium acetate in anhydrous N,N-dimethylformamide. After 20 minutes, the reaction was worked-up and the crude product was chromatographed on a column of silica gel, which was packed and eluted with 1:1 benzene-ethyl acetate, to afford the ketose 25 (R$_f$ 0.44) and a pale yellow syrup (R$_f$ 0.58) which was shown, by i.r. and n.m.r. spectroscopy, to be composed of methyl nitroacetate and the β-hydroxy-α-nitro ester 162.

In the p.m.r. spectrum of the syrup (Figure 3) the presence of methyl nitroacetate was evidenced by a methyl ester resonance at δ3.88 and a methylene proton signal at δ5.18. The existence of compound 162 was attested to by the presence of;
Figure 3  Partial 100 MHz P.M.R. Spectrum of 1,2:5,6-di-O-Isopropylidene-3-C-[(R,S)-nitro(methoxycarbonyl)methyl]-α-D-allofuranose (162) in CDCl₃.
1) a one proton singlet at δ5.83 attributed to the single branch-chain proton
2) a three proton singlet at δ3.83 attributed to the methyl ester, and
3) a broad one proton signal at δ3.47 - which disappeared upon addition of D₂O - attributed to the tertiary hydroxyl proton at C-3.

It was calculated, from the relative intensities of the resonances, that the product was a 5:1 mixture of compound 162 and methyl nitroacetate.

The i.r. spectrum of the syrup exhibited absorptions of hydroxyl (3450 cm⁻¹), ester (1755 cm⁻¹), and nitro groups (1570 cm⁻¹) in support of the n.m.r. evidence.

In an attempt to isolate compound 162, the product mixture was rechromatographed on a column of silica gel using 8:2 benzene-ethyl acetate as the developer. Surprisingly, the only carbohydrate material recovered was the diisopropylidene ketose 25. All other solvent mixtures employed in an attempt to isolate compound 162 by column chromatography or preparative t.l.c. resulted in either no separation of the product mixture or decomposition of the nitro ester 162 into the starting materials.
3.2.2 Synthesis of 3-0-acetyl-1,2:5,6-di-0-isopropylidene-3-C-[(R,S)nitro(methoxycarbonyl)methyl]-α-D-allofuranose (165).

It was decided that the best way to isolate the condensation product was to "trap" it by derivatizing the hydroxyl group and thereby prevent the decomposition back into starting materials. Acid catalysed acetylation was the method of derivatization chosen due to the tertiary nature of the hydroxy group involved.

Thus, the reaction of ketose 25 and methyl nitroacetate was carried out in the usual way up to the chromatography. The partially purified and dried product mixture was then dissolved in acetic anhydride and a catalytic amount (10-15% w/w) of p-toluenesulfonic acid monohydrate was added to the solution. After heating at 80-85°C for 5 hours, the reaction mixture was worked-up and the black product mixture was chromatographed to yield a pale yellow syrup which crystallized spontaneously upon standing. Recrystallization of the crude product afforded the 3-0-acetyl nitro ester 165 in 82% yield.

The p.m.r. spectrum of compound 165 (see Figure 4) clearly shows the singlets of the 0-acetate (δ2.08), the methyl ester (δ3.80) and the branched-chain proton (δ5.90) and the presence of the nitro group was confirmed by an absorption in the i.r. spectrum at 1570 cm⁻¹. The
Figure 4  60 MHz P.M.R. Spectrum of 3-O-Acetyl-1,2:5,6-di-O-n-propylidene-3-C-[(R,S)-nitro(methoxycarbonyl)methyl]-α-D-allofuranose (165) in CDCl₃.
elemental analysis of compound 165 was also consistent with its proposed structure.

\[
\text{Ac}_2\text{O} \quad \frac{\text{p-TSA} \cdot \text{H}_2\text{O}}{80-85^\circ/5\text{h}}
\]

3.3 Reduction and Proof of Structure of the Methyl Nitroacetate Adducts.

The reduction of the glycosyl α-nitro esters 162 and 165 would not only place the synthesis of polyoxin analogues one step nearer, it would also provide a means of determining the absolute configuration at C-3 of the resulting glycosyl α-amino esters. This is possible because Rosenthal et al.\textsuperscript{178,87} have previously synthesized glycos-3-yl amino acids having the gluco configuration for which an unequivocal proof of structure has been provided and therefore the configuration of the amino esters derived from the nitro esters can be determined by direct comparison with authentic samples. The compounds which are best suited
for such a comparison are $L$-2 and $D$-2-(1,2:5,6-di-0-isopropylidene-$\alpha$-$D$-glucofuranos-3-yl)glycine (200 and 201, respectively) and their corresponding $N$-acetyl methyl esters (202 and 203, respectively).

3.3.1 Synthesis of Methyl $N$-acetyl-$L$ and $D$-2-(1,2:5,6-di-0-isopropylidene-$\alpha$-$D$-glucofuranos-3-yl)glycinate (202 and 203).

Palladium on charcoal$^{200,97}$, platinum oxide$^{95}$, 201-203 and Raney nickel$^{186-204}$ have been routinely used to reduce nitro functionalities on sugars. When the palladium or platinum catalysts were used in attempts to reduce the mixture of the $\beta$-hydroxy $\alpha$-nitro ester$^{162}$ and methyl nitroacetate, in a methanolic medium, a very
complex mixture composed of a great number of components was produced. Even after repeated chromatography no one pure compound could be separated or identified. However, when the reduction was repeated using palladium on charcoal in the presence of acetic anhydride, and the resulting crude product mixture was chromatographed on a column of silica gel using 10:5:1 benzene-ether-ethanol as the developer, the \( \text{N-acetyl } \text{L-amino ester 202} \) (12%) and the \( \text{N-acetyl } \text{D-amino ester 203} \) (9%) were isolated along with ketose 25 and a complex mixture of at least nine unidentified compounds. Compounds 202 and 203 were identical (p.m.r., optical rotation) to authentic samples previously prepared. However, due to the complex nature of the product mixture, and because of the low yields of the identifiable components, no safe inference could be made about the configuration at \( \text{C-3} \) of the nitro ester 162.

3.3.2 Synthesis of \( \text{3-0-Acetyl-1,2:5,6-di-0-isopropylidene-3-C-}(\text{methoxydicarbonyl})-\alpha-\text{D-allofuranose oxime (204).} \)

Hydrogenation of a methanolic solution of the 3-0-acetyl nitro ester 165 over palladium on charcoal and under an atmosphere of hydrogen for 48 hours afforded one chromatographically pure compound in 94% yield. The characteristic absorptions of an amine were absent from both the i.r. and p.m.r. spectra of the product. Instead,
the p.m.r. spectrum showed only one exchangeable proton as a broad signal between δ5.5 - 4.9. From the elemental analysis of the product the molecular formula of the product was calculated to be C_{17}H_{25}NO_{10}. The integration of the p.m.r. spectrum agrees exactly with the presence of twenty-five protons and it was thus concluded that the reduction of compound 165 had proceeded only as far as the oxime 204.

3.3.3 Synthesis of Methyl L- and D-2-(1,2:5,6-di-O-
isopropylidene-α-D-allofuranos-3-yl)glycinate (205 and 206).

Hydrogenation of the 3-O-acetyl nitro ester 165 over freshly prepared Raney nickel W-4 catalyst 207 afforded, after column chromatography, a major component 205 (67%) and a minor component 206 (8%), both of which were faintly ninhydrin positive. The decreased intensity of the colour reaction compared to that exhibited by amino acids is characteristic of α-amino esters 87,178,179,196. Compounds 205 and 206 also have p.m.r. spectra typical of α-amino esters; i.e.

1) the broad, high field resonance (δ 1.83 and δ2.08 respectively) of the two amine protons which was easily identified since it rapidly disappeared upon addition of deuterium oxide, and
2) the broad, lower field resonance (δ 4.11 and δ 4.02, respectively) of the branch-chain proton which, after addition of deuterium oxide, immediately collapsed to a sharp singlet.

When the previously obtained oxime 204 was hydrogenated in the presence of Raney nickel catalyst, it afforded a product mixture identical in composition to that obtained by reduction of compound 165. In fact, when reduction of compound 165 was interrupted by removing the catalyst before all of the starting material was consumed, the chromatography of the resulting mixture of compounds on a column of silica gel, using 9:1 ethyl acetate-ether as the developer, afforded the starting material 165, the oxime 204 and the α-amino esters 205 and 206.

3.3.4 Synthesis of $L$- and $D$-2-(1,2:5,6-Di-O-isopropylidene-$D$-allofuranos-3-yl)glycine (207 and 208).

The alkaline hydrolysis of compounds 205 and 206 provides a ready proof of structure by enabling one to determine 1) the absolute configuration of the α-carbon of the amino acid by circular dichroism and 2) the absolute configuration at C-3 by direct comparison with the previously described gluco-α-amino acids 200 and 201.

Thus, compound 205 was treated with methanolic sodium hydroxide and after 4 hours at room temperature the solution was applied to a short column of Rexyn RG-51 ($H^+$) resin in order to remove the sodium ions. The column was eluted with water and those fractions which gave a strong
positive ninhydrin reaction were collected and evaporated to yield the free α-amino acid 207 in an 82% yield. The circular dichroism spectrum of compound 207 in 0.5 N methanolic hydrochloric acid exhibited a positive Cotton effect (Δε + 0.97) at 209 nm. Compound 207 was therefore assigned the L-amino acid configuration in agreement with the positive Cotton effect exhibited by other L-amino acids178,179,208.

TABLE 4. Comparison of Physical Constants of Various Glycos-3-yl Amino Acids.

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>m.p.(°C)</th>
<th>[α]D</th>
<th>c.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>207</td>
<td>189-191</td>
<td>+89.2°</td>
<td>Δε209 +0.97</td>
</tr>
<tr>
<td>200</td>
<td>185.5-186.5</td>
<td>+51.1°</td>
<td>Δε212 +1.55</td>
</tr>
<tr>
<td>208</td>
<td>157-159</td>
<td>+25.0°</td>
<td>Δε209 -0.93</td>
</tr>
<tr>
<td>201</td>
<td>193.5-195.0</td>
<td>+35.0°</td>
<td>Δε212 -1.41</td>
</tr>
<tr>
<td>211</td>
<td>141-142</td>
<td>+27.4°</td>
<td></td>
</tr>
<tr>
<td>202</td>
<td>52-62</td>
<td>+71.0°</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(glass)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>212</td>
<td>syrup</td>
<td>+45.9°</td>
<td></td>
</tr>
<tr>
<td>203</td>
<td>54-56</td>
<td>+50.6°</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(glass)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
An examination of the physical characteristics of the L-amino acids 200 and 207, (as shown in Table 4), reveals that significant differences exist between them. A significant melting point depression was also observed for a mixture of the two compounds. It is therefore proposed that compounds 207 and 200 are diastereomers differing in configuration at C-3 of the sugar and that compound 207 is L-2-(1,2:5,6-di-O-isopropylidene-α-D-allofuranos-3-yl) glycine. Compound 205 is thus designated methyl L-2-(3-O-acetyl-1,2:5,6-di-O-isopropylidene-α-D-allofuranos-3-yl) glycinate.

The hydrolysis of the amino ester 206 afforded the α-amino acid 208 in a 85% yield. The circular dichroism spectrum of a methanolic 0.5 N hydrochloric acid solution of compound 208 shows a negative Cotton effect (Δε -.93) at 209 nm and compound 208 is therefore assumed to possess a D-amino acid configuration. Table 4 shows that significant differences exist in the physical characteristics of D-amino acids 208 and 201 and on this basis it is proposed that compound 208 is D-2-(1,2:5,6-di-O-isopropylidene-α-D-allofuranos-3-yl)glycine. It therefore follows that the α-amino ester 206 must be methyl D-2-(3-O-acetyl-1,2:5,6-di-O-isopropylidene-α-D-allofuranos-3-yl)glycinate.

In order to provide another basis for the comparison of the gluco and allo amino acids, the syntheses
of methyl L-acetyl-\(\text{L}\) and D-2-(1,2:5,6-di-\(\text{O}\)-isopropylidene-\(\text{\(\alpha\)}\)-D-allofuranos-3-yl)glycinate (211 and 212) were undertaken. The L-amino ester 205 was acetylated using acetic anhydride in methanol to afford, in quantitative yield, a compound whose p.m.r. spectrum and elemental analysis are consistent with its designation as methyl N-acetyl-L-2-(3-\(\text{O}\)-acetyl-1,2:5,6-di-\(\text{O}\)-isopropylidene-\(\text{\(\alpha\)}\)-D-allofuranos-3-yl)glycinate (209). To hydrolyze the \(\text{O}\)-acetate group, a catalytic amount of sodium methoxide was added to a solution of compound 209 in methanol. After 25 minutes the solution was de-cationized with Amberlite IRC-50 (\(\text{H}^+\)) resin and evaporated to afford a glass. The crude product was chromatographed on silica gel to afford pure compound 211 in 91\% yield.

The p.m.r. spectrum of the product shows an acetate methyl signal at \(\delta 2.05\) and a broad NH resonance at \(\delta 6.83\) typical of amides. The NH resonance exists as a doublet and is coupled to the H-1 doublet at \(\delta 5.22\) with a coupling constant of 9.5 Hz. With the addition of deuterium oxide to the sample, the amide resonance disappeared, the H-1' signal collapsed to a singlet, and a broad singlet at \(\delta 3.09\), attributed to the C-3 hydroxyl, disappeared. In addition to the p.m.r. spectrum, the elemental analysis is also in agreement with the proposed structure of compound 211.
The acetylation of compound 206 and the subsequent hydrolysis of the O-acetate of the intermediate methyl N-acetyl-D-2-(3-0-acetyl-1,2:5,6-di-O-isopropylidene-α-D-allofuranos-3-yl)glycinate (210) yielded methyl N-acetyl-D-2-(1,2:5,6-di-O-isopropylidene-α-D-allofuranos-3-yl)-glycinate (212) in an overall 64% yield.

In the p.m.r. spectrum of compound 212 the NHAc proton appears as a broad doublet at δ7.08 coupled to the H-1' proton resonance at δ5.25 with a J value of 10.1 Hz, and the free C-3 hydroxyl is shown as a broad singlet at δ2.54.

Table 4 shows that the N-acetyl amino esters 211 and 212 and the gluco N-acetyl amino esters 202 and 203 have distinctly different physical characteristics, melting points, and optical rotations. This, together with the dissimilar p.m.r. spectra (shown in Figures 5 and 6), leaves no doubt that the four compounds are diastereomers and is further support of the allo configuration assigned to compounds 211 and 212.

In section 3.3.1 it was reported that the reduction of the β-hydroxy α-nitro ester 162 over palladium on charcoal, in the presence of acetic anhydride, afforded a myriad of products and that the only two products identified were of the gluco configuration. This apparent contradiction can be explained by postulating that under the conditions employed for the reduction, an equilibrium
Figure 5  100 MHz P.M.R. Spectrums of Methyl N-acetyl-L-2-(1,2-0-isopropylidene-α-D-gluco and allofuranos-3-yl)glycinate (202 and 211) in CDCl₃.
Figure 6  100 MHz P.M.R. Spectrums of Methyl N-acetyl-\(\beta\)-2-(1,2-\(\alpha\)-isopropylidene-\(\alpha\)-\(\beta\)-gluco and alolfuranos-3-yl)glycinate (203 and 212) in CDCl\(_3\).
exists between the allo and gluco condensation products.

It has been previously demonstrated that the condensation product will revert back into ketose and methyl nitroacetate under certain chromatographic conditions so the condensation must be regarded as a reversible reaction until acid catalyzed acetylation of the condensation product "traps" it in the allo configuration as the proof of structure has shown.

3.4 Oxidative Cleavage of Methyl N-acetyl-L-2-(1,2-0-isopropylidene-α-D-allofuranos-3-yl)glycinate (226) with Sodium Periodate.

The periodate cleavage of the 5,6-vicinal diol of a 3-C-aminomethyl-allofuranose branched-chain sugar often results in spontaneous condensation of the amine functionality with the resulting 5-aldehyde to yield a 5-membered
cyclic aminal. This reaction was used to prove the cis relationship between the 5,6 "tail" and the 3-C-acetamino-methyl branch chain of compound 213. Jordaan and co-

\[
\begin{align*}
\text{HO} & \quad \text{HO} \\
\text{NR}_1 & \quad \text{CHR} \\
\text{CHR} & \quad \text{HO} \\
\text{HO} & \quad \text{O} \\
\text{R}_1 & \quad \text{O} \\
\end{align*}
\]

\[
\begin{align*}
213 & \quad R = H; \quad R_1 = \text{Ac} \\
214 & \quad R = \text{CO}_2\text{Et}; \quad R_1 = \text{CHO} \\
215 & \quad \text{R} \\
216 & \quad \text{R} \\
217 & \quad \text{R} \\
\end{align*}
\]

workers encountered a similar reaction when they attempted to cleave and reduce the 5,6 diol 214. Similarly Rosenthal and Ratcliffe proved the allo stereochemistry of their 3-C-carbamoyl sugar 216 by formation of the aminal 217.
In order to provide additional confirmation of the \textit{allo} configuration of the \textalpha-amino ester 205, it was decided to attempt such an aminal synthesis.

### 3.4.1 Synthesis of Methyl \textit{N}-acetyl-L-2-(1,2-\textO-isopropylidene-\textalpha-D-allofuranos-3-yl)glycinate (226)

The synthetic strategy originally envisioned for the synthesis of an aminal involved:

1) hydrolysis of the 5,6-\textO-isopropylidene group of the fully blocked glycosyl amino acid 209 to afford the 5,6 diol 218,
2) oxidative cleavage of the diol 218 to afford the 5-aldehydo sugar 219, and
3) spontaneous cyclization of compound 219 to afford the aminal 220.
Towards this end, compound 209 was dissolved in 66% acetic acid and after 24 hours at room temperature the solvent was removed to afford a product which crystallized spontaneously. The main features of the p.m.r. spectrum of the product in chloroform-\textsubscript{d} were:

1) two acetate methyl resonances at \( \delta 2.08 \) and \( \delta 2.06 \),
2) a methyl ester absorption at \( \delta 3.75 \)
3) a typical broad doublet of the amide proton at \( \delta 7.23 \) coupled to the branch-chain proton at \( \delta 5.23 \) with \( J \) equal to 8.0 Hz, and
4) two broad single proton signals at \( \delta 4.69 \) and \( \delta 4 \) which exchanged in D\textsubscript{2}O and were assigned to the C-5 and 6 hydroxyl protons.

The elemental analysis of the product suggested a molecular formula of \( \text{C}_{16}\text{H}_{25}\text{NO}_{10} \) and on the basis of this and the p.m.r. spectrum the product was designated methyl N-acetyl-L-2-(3-O-acetyl-1,2-O-isopropylidene-\( \alpha \text{-D-} \)allofuranos-3-yl)glycinate (218).

Surprisingly, treatment of the crystalline product, obtained from the hydrolysis of compound 209, with sodium metaperiodate under standard oxidative-cleavage conditions yielded the starting material unchanged. It was also observed that when the hydrolysis product was acetylated, using acetic anhydride and pyridine, the sole product isolated (compound 222) had an elemental analysis
and p.m.r. spectrum consistent with a compound possessing a methyl N-acetyl glycinate branch-chain, two O-acetate groups and one free hydroxyl. The remaining free hydroxyl group of compound 222 was finally acetylated using acetic anhydride and p-toluenesulphonic acid monohydrate at elevated temperature to yield the fully blocked methyl N-acetyl-\(\alpha\)-2-(3,5,6-tri-O-acetyl-1,2-O-isopropylidene-\(\alpha\)-D-allofuranos-3-yl)glycinate (223).

From these reactions it seemed obvious that the hydrolysis of compound 209 did not result in formation of the diol 218 but that instead the hydrolysis was followed by migration of the 3-O-acetyl group to either the C-5 or C-6 hydroxyl to yield compound 221.

Acyl migrations in carbohydrate chemistry have been well documented\(^{206-208}\). Although the allo stereochemistry at first seemed prohibitive, the construction of
a molecular model showed that the ortho-ester intermediate \(224\) could be easily attained from compound \(218\). Since the movement of the acyl group is, in the majority of cases, towards a primary hydroxyl group \(208\), it was felt that compound \(221\) was probably the product of an initial 3-0 to
5-0 migration followed by a 5-0 to 6-0 migration. In order to confirm this suspicion the p.m.r. spectrum of compound 221 in dimethyl sulfoxide-$d_6$ was obtained (see Figure 7). The tertiary C-3 hydroxyl proton was evident as a singlet at $\delta 5.46$. At $\delta 5.28$ was a doublet with a coupling constant of 5.2 Hz which was attributed to the C-5 hydroxyl proton. Both of these resonances disappeared upon addition of D$_2$O to the sample. On the basis of this evidence compound 221 was assigned as methyl N-acetyl-L-$\psi$-2-(6-0-acetyl-1,2-0-isopropylidene-$\alpha$-$D$-allofuranos-3-yl) glycinate.

It was obvious that prior to oxidative cleavage of the 5,6 "tail" the 6-0-acetyl group of compound 221 would have to be removed. This was accomplished by treating a methanolic solution of 221 with a catalytic amount of sodium methoxide, in a reaction identical to that employed in the de-acetylation of compound 209, to yield methyl N-acetyl-L-$\psi$-2-(1,2-0-isopropylidene-$\alpha$-$D$-allofuranos-3-yl)glycinate (226) in a 95% yield. Alternately compound 226 was prepared by acid hydrolysis of the 5,6-isopropylidene group of compound 211.

The triol 226 was characterized as its di-acetate 222 in that acetylation of 226 using acetic anhydride and pyridine produced a compound that was identical ([a]$_D$, p.m.r., i.r.) to the product of the acetylation of compound 221.
Figure 7 100 MHz P.M.R. Spectrum of Methyl N-acetyl-L-2-(6-O-acetyl-1,2-O-isopropylidene-α-D-allofuranos-3-yl)glycinate (221) in DMSO-d$_6$. 
3.4.2 Attempted Synthesis of 3-C-[(S)-Acetamino-(carbomethoxy)methyl]-1,2-0-isopropylidene-α-D-ribo-pentodialdofuranose-5,N-aminal (228).

Compound 226 was dissolved in a 2:1 methanol-water solution and to this was slowly added a concentrated water solution of sodium metaperiodate. After the addition of 1.1 equivalents of periodate the t.l.c. of the reaction showed that all of the starting material (Rf 0.21, 8:2 benzene-ethanol) had been consumed and only one other spot (Rf 0.40) was evident. Work-up of the reaction yielded a colourless glass.

It was assumed that, similar to previous examples, the product of the periodate reaction
was the aldehydo-sugar 227 which was in equilibrium with
the aminal 228. In an attempt to isolate the aminal, as
its 5-O-acetyl derivative 229, the crude product of the
reaction was dissolved in a solution of acetic anhydride-
pyridine for 24 hours. Chromatography of the product of
the acetylation, however, afforded only a mixture of at
least five impure components - none of which could be
reasonable assigned a structure or further purified easily.
Attempts to isolate the di-acetate aminal 230 or the di-
benzoyl aminal 231 by acid catalysed acetylation or
benzoylation, respectively, of the crude product of the
periodate reaction produced similar complex mixtures of
products. The attempt to synthesize an aminal from com-
pound 226 was therefore abandoned.

3.4.3 Synthesis of Methyl N-acetyl-L-2-(1,2-0-
isopropylidene-a-D-ribofuranos-3-yl)glycinate
(232) and Methyl N-acetyl-L-2-(5-O-acetyl-1,2-
0-isopropylidene-a-D-ribofuranos-3-yl)glycinate
(233).

In order to ensure that the oxidative cleavage
of compound 226 was producing the expected product, namely
the 5-aldehydo sugar 227, the reaction was repeated and
after t.l.c. had shown all the starting material to be
consumed an excess of sodium borohydride was added. After
15 minutes the reaction was worked-up to yield a colourless
glass. This product was not characterized but was immediately dissolved in acetic anhydride and pyridine for 24 hours in an attempt to acetylate the primary hydroxyl group produced by the reduction. Chromatography of the crude material from the acetylation on a column of silica gel, using 8:2 benzene-ethanol as developer, afforded two products 232 and 233.

\[
\begin{align*}
\text{HOH}_2C & \quad \text{CO}_2\text{Me} \\
\text{AcN} & \quad \text{H} \\
\text{HO} & \quad \text{O} \\
\text{O} & \quad \text{O}
\end{align*}
\]

The p.m.r. spectrum of the lower \( R_f \) component (26\%) showed the presence of only one acetate methyl resonance and that was attributed to the \( \text{N}-\)acetyl group. Also evident were the resonances of three exchangeable protons. The low field doublet at \( \delta 6.95 \) was, of course, attributed to the amide proton. The second exchangeable proton appeared as a broad singlet at \( \delta 5.57 \) while the third was hidden under the overlapping signals (\( \delta 4.60 - \delta 3.95 \)) of four other protons. The presence of this last proton was assumed from the reduction of the integrated
area of the signals between $\delta 4.60$ and $\delta 3.95$ from an equivalent of five protons to four protons upon addition of D$_2$O to the sample. The second and third exchangeable protons were assumed to be hydroxyl protons. In all, the p.m.r. spectrum indicated the presence of 21 protons consistent with the structure of the pentofuranosyl diol 232.

The high resolution mass spectrum of the lower $R_f$ component was also consistent with it being the diol 232. An intense peak in the spectrum occurred at a mass-to-charge ratio of 304.1013 which corresponded to the mass fragment arising from loss of a methyl group from the parent ion $C_{13}H_{21}NO_8$. The theoretical value for a mass of $C_{12}H_{18}NO_8$ was 304.1032. The elemental analysis of the component also agreed with the assigned structure.

The higher $R_f$ material gave physical and spectral evidence consistent with its structural assignment as the 5-O-acetyl sugar 233. The p.m.r. spectrum indicated the presence of one O-acetate and one free hydroxyl group in addition to the N-acetyl amino ester branch-chain. The high resolution mass spectrum showed a parent peak at an m/e value of 261.1349 and a much more intense peak, corresponding to loss of CH$_3$, at 346.1136. The theoretical values for masses of $C_{15}H_{23}NO_9$ and $C_{14}H_{20}NO_9$ were 361.1372 and 346.1138, respectively. Finally, the elemental analysis was consistent with a molecular composition of $C_{15}H_{23}NO_9$.

Quantitative conversion of the diol 232 into its 5-O-acetyl derivative 233 was accomplished by continuing the acetylation reaction for an additional 48 hours.
3.5 Synthesis of 3-Deoxy-3-C-nitro(methoxycarbonyl)methyl allofuranoses.

Although, in our synthesis of an analogue of the polyoxins, our prime objective was the synthesis of a glycos-3-yl amino acid in which the 3-C-hydroxyl group was retained, we were also interested to see if the methyl nitroacetate condensation could provide a route to the 3-deoxy-3-C-glycosyl amino acids 177, 181, 182.

3.5.1 Attempted Synthesis of 5,6-Di-O-acetyl-3-C-[acetamino(methoxycarbonyl)methylene]-3-deoxy-1,2-O-isopropylidene-\(\alpha\)-D-ribo-hexofuranose (234).

The initial attempt to synthesize a 3-deoxy sugar involved the dehydration of the \(\beta\)-hydroxy-\(\alpha\)-acetamido ester 222. Freshly distilled thionyl chloride was added to a pyridine solution of compound 222 (kept at \(-25^\circ\)) and the mixture was maintained at \(5^\circ\) overnight. Work-up of the reaction mixture yielded a dark amber syrup which was prone to decomposition at room temperature either in solution or neat. On the assumption that the product was the unsaturated acetamido ester 234 the product was immediately dissolved in methanol and hydrogenated over Raney nickel for 24 hours. The solution was then filtered and evaporated and the resulting syrup was chromatographed on a column of silica gel to afford a
single product (29%). Quite unexpectedly, the product was shown (p.m.r. \([\alpha]_D\), i.r.) to be the starting compound 222.

One possible explanation for the observed results, in the absence of physical or spectroscopic evidence, is that the compound isolated after the initial "dehydration" reaction was an intermediate alkyl chlorosulfite (ROSOC1). Such compounds have been isolated\(^\text{221}\). Decomposition of the unstable chlorosulfite would undoubtedly cause further degradation of the acid sensitive carbohydrate and would account for the observed instability of the isolated compound at room temperature. In the presence of Raney nickel, however, the alkyl chlorosulfite was evidently hydrolyzed to yield the original alcohol.
3.5.2 Synthesis of 3-Deoxy-1,2:5,6-di-0-isopropylidene-3-C-[(R,S)-nitro(methoxycarbonyl)methyl]-α-D-allofuranose (236).

As previously described in section 3.1, the condensation of methyl nitroacetate with the 5-0-benzoyl-pentos-3-ulose 182 gave a mixture of the simple addition product 187 and the unsaturated nitro ester 189. The acid catalyzed acetylation conditions used in an attempt to isolate compound 187 as its 3-0-acetyl derivative resulted, instead, in dehydration of compound 187 to afford the unsaturated compound 189 as the sole product. It was therefore quite surprising to find that an identical series of reactions using the ketose 25 afforded only the 3-0-acetyl derivative 165 with no trace of the 3-deoxy-1,2:5,6-di-0-isopropylidene-3-C-[nitro(methoxycarbonyl)methylene]-α-D-ribo-hexofuranose (235). However, when the acetylation was performed for one hour at 120°C, the chromatography of the black syrup obtained upon work-up of the reaction afforded the β-acetoxy nitro ester 165 (R_f 0.38 in 8:2 benzene-ethyl acetate, 63%) and the α,β-unsaturated nitro ester 235 (R_f 0.42, 4%).

The p.m.r. spectrum of compound 235 was complex and showed a mixture of two components in an approximate ratio of 1.5:1 as calculated from the relative heights of the methyl ester resonances at δ3.94 and 3.98, respectively. The H-2 protons appeared as superimposed quartets at
65.50. In the major isomer H-2 was coupled to the anomeric proton (δ5.89) and H-4 (δ5.69) with coupling constants of 5.0 Hz and 2.0 Hz, respectively, while in the minor isomer H-2 was coupled to H-1 (δ5.87) with \( J_{1,2} = 4.8 \) Hz and H-4 (δ5.62) with \( J_{2,4} = 2.0 \) Hz. As can be seen in Table 5, the low field region of the p.m.r. spectrum of compound 235 is very similar to that of the previously described \( \alpha,\beta \)-unsaturated nitro esters 189 and 190.

Compound 235 was not characterized but was immediately reduced to afford the 3-deoxy-3-\( \text{C} \)-nitro-(methoxycarbonyl)methyl sugar 236.

### Table 5

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>H-1 (δ)</th>
<th>( J_{1,2} ) (Hz)</th>
<th>H-2 (δ)</th>
<th>( J_{2,4} ) (Hz)</th>
<th>H-4 (δ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>189(^a)</td>
<td>6.02</td>
<td>4.8</td>
<td>5.52</td>
<td>1.8</td>
<td>6.00</td>
</tr>
<tr>
<td>189(^b)</td>
<td>6.05</td>
<td>4.5</td>
<td>5.67</td>
<td>1.8</td>
<td>5.86</td>
</tr>
<tr>
<td>190</td>
<td>5.98</td>
<td>4.8</td>
<td>5.48</td>
<td>1.5</td>
<td>5.73</td>
</tr>
<tr>
<td>235(^a)</td>
<td>5.89</td>
<td>5.0</td>
<td>5.50</td>
<td>2.0</td>
<td>5.69</td>
</tr>
<tr>
<td>235(^b)</td>
<td>5.87</td>
<td>4.8</td>
<td>5.50</td>
<td>2.0</td>
<td>5.62</td>
</tr>
</tbody>
</table>

\(^a\) - denotes major isomer. \(^b\) - denotes minor isomer
Sodium borohydride has been used to reduce double bonds of \( \alpha,\beta \)-unsaturated esters\(^{209}\) and nitroalkenes\(^{210}\). For the latter class of compounds, however, the reaction required that the pH of the medium be maintained between the limits 3-6 in order to inhibit the formation of the \( \alpha \)-carbon in the nitroalkane and retard Michael addition to the nitroalkene which would result in dimeric products\(^{210-211}\). For this reason the more acid stable sodium cyanoborohydride\(^{212}\) was used in reduction of compound 235 and the pH of the methanolic medium was maintained between 3-4 by the periodic addition of 1% hydro-
chloric acid in methanol. Compound 236 was thus obtained in a 90% yield from 235.

The stereochemistry at C-3 was deduced from the p.m.r. spectrum (see Figure 8). As in the case of the spectrum of the unsaturated ester 235, the spectrum of compound 236 showed the presence of two components. Stereochemically, the most important resonances were those of the H-2 protons and that of the major product, designated A, appeared as a triplet at $\delta 5.09$ while that of the minor product, designated B, appeared as a triplet at $\delta 4.87$. A component ratio of A:B equal to 2.2:1 was calculated from the integrated areas of the H-2 resonances. Protons H-1A and H-1B existed as a single doublet at $\delta 5.84$ with $J_{1,2} = 3.8$ Hz. Irradiation of H-1 caused the H-2A and H-2B signals to collapse to doublets with $J_{2,3} = 4.9$ Hz. It has been amply demonstrated$^{213,177,179}$ that H-2, H-3 coupling constants of this magnitude in 3-deoxy-1,2-0-isopropylidene-hexofuranose systems are indicative of allo-stereochemistry and therefore, compound 236 must be 3-deoxy-1,2:5,6-di-0-isopropylidene-3-C-[(R,S)-nitro(methoxy-carbonyl)methyl]-\(\alpha\)-D-allofuranose.

As a further proof of structure, compound 236 was converted into a number of previously synthesized compounds of known stereochemistry.

Reduction of the nitro group of 236 by hydrogenation over Raney nickel catalyst afforded the \(\alpha\)-amino
Figure 8  Partial 100 MHz P.M.R. Spectrum of 3-Deoxy-1,2:5,6-di-0-isopropylidene-3-C-[(R,S)-nitro(methoxycarbonyl)methyl]-α-D-allofuranose (236) in CDCl₃.
esters 141 (46%) and 142 (38%). Compound 141 exhibited a p.m.r. spectrum superimposable with the spectrum of the previously described methyl \( \text{L}-2-(3\text{-deoxy-1,2:5,6-di-0-isopropylidene-}\alpha-\text{D-allofuranos-3-yl})\text{glycinate} \) while compound 142 had a p.m.r. spectrum identical with the corresponding D-glycinate. Furthermore, when the \( \text{L}- \) amino ester 141 was hydrolyzed with a methanol-water solution of sodium hydroxide (0.6%) and the product of the reaction was passed through a column of RG-51 (\( \text{H}^+ \)) cation exchange resin, the white crystalline material isolated (62%) possessed a melting point and optical rotation which indicated it was \( \text{L}-2-(3\text{-deoxy-1,2-0-isopropylidene-}\alpha-\text{D-allofuranos-3-yl})\text{-glycine} \) (237). Benzoylation of the D-amino ester 142 yielded a compound identical (m.p., \([\alpha]_D\)) with methyl N-benzoyl-D-2-(3-deoxy-1,2:5,6-di-0-isopropylidene-\(\alpha-\text{D-}
\)
allofuranos-3-yl)glycinate (238).

\[ \begin{align*}
236 & \xrightarrow{\text{Raney Ni, } H_2} \\
141 & R=H; R_1=NH_2 \\
142 & R=NH_2; R_1=H \\
238 & R=NHCOC_6H_5; R_1=H \\
237 & \\
\end{align*} \]
As previously described (see the Introduction, section 5) an unambiguous confirmation of the configurational assignments of these glycosyl \( \alpha \)-amino acid derivatives, synthesized by Rosenthal and co-workers\(^{177-179,184}\), was provided\(^{179}\) by converting the \( \alpha \)-amino ester \(^{142}\) into 3-deoxy-3-C-[(\( R \))-hydroxymethyl-(\( N \)-salicylideneamino)-methyl]-1,2:5,6-di-O-isopropylidene-\( \alpha \)-\( \alpha \)-allofuranose (148), a previously reported compound\(^{180}\) whose structure was correlated with 5-O-(\( p \)-bromophenylsulphonyl)-3-deoxy-3-C-(\( R \)-)(ethoxycarbonylformamido)methyl-1,2-O-isopropylidene-\( \alpha \)-\( \alpha \)-ribofuranose (147)\(^{180}\). The structure of compound 147 has been determined by X-ray analysis\(^{180,183}\).
3.5.3 Synthesis of 5,6-Di-O-acetyl-3-deoxy-1,2-O-isopropylidene-3-C-[(R,S)-nitro(methoxycarbonyl)methyl]-α-D-allofuranose (241).

It seemed obvious that a necessary requirement for the acid catalyzed generation of the unsaturated nitro ester 235, from compound 162, was the presence of a free tertiary hydroxyl group at C-3. This was confirmed by an experiment in which the β-acetoxy-α-nitro ester 165 was recovered unchanged after being heated for four hours at
120°C in acetic anhydride containing p-toluenesulphonic acid monohydrate (15% w/w with respect to 165).

In section 3.4.1 we reported the 0-3 to 0-6 migration of an acyl group during the acid hydrolysis of a 5,6-0-isopropylidene blocking group. It was felt that if such a migration took place during the hydrolysis of compound 165 then the resulting β-hydroxy-α-nitro ester 239, would be susceptible to acid catalyzed dehydration to afford the di-0-acetyl unsaturated nitro ester 240.

\[
\begin{align*}
165 & \xrightarrow{\text{H}_2\text{O}^+} \quad \text{Ac} & \quad \text{CO}_2\text{Me} \\
 & \quad \text{H} & \quad \text{CHNO}_2 \\
 & \quad \text{HO} & \quad \text{Ac} \\
239 & \quad \text{Ac} & \quad \text{Ac} \\
 & \quad \text{p-TSA} & \quad \text{O}_2\text{N} \quad \text{CO}_2\text{Me} \\
240 & \quad \text{O} & \quad \text{O} \\
& \quad \text{O} & \quad \text{O}
\end{align*}
\]

Compound 165 was dissolved in 66% acetic acid and heated for five hours at 50°. The acetic acid was then evaporated and the crude product decolourized with activated charcoal to afford a clear colourless syrup which crystallized spontaneously upon standing. The elemental analysis of a recrystallized sample was consistent with the desired molecular formula of C_{14}H_{21}NO_{11}.
The p.m.r. spectrum of the sample was obtained in dimethyl sulfoxide-d$_6$ in an attempt to determine the location of the acetate group by the degree of splitting exhibited by the hydroxyl protons; however, no definite conclusions could be drawn. There were five resonances in the low field region between $\delta$5 and $\delta$7 which integrated to a total of four protons. The branch-chain proton appeared as a singlet at $\delta$6.22 and the anomeric proton signal was a doublet at $\delta$5.84 with $J_{1,2} = 4.0$ Hz. The three remaining resonances - a sharp singlet at $\delta$6.44 equivalent to one proton, a sharp singlet at $\delta$5.70 equivalent to $\sim 1/2$ proton, and a broad singlet at $\delta$5.51 equivalent to $\sim 1/2$ proton - all disappeared after addition of deuterium oxide to the sample and were attributed to the two hydroxyl protons of the diastereomeric mixture. That the compound was a mixture of two components was further evidenced by the existence of two methyl ester peaks at $\delta$3.79 and $\delta$3.74. Compound 239 was therefore designated 3-, 5-, or 6-0-acetyl-1,2-0-isopropylidene-3-C-[(R,S)-nitro-(methoxycarbonyl)methyl]-a-D-allofuranose.

Compound 239 and p-toluenesulphonic acid monohydrate (13% w/w) were dissolved in acetic anhydride and the solution was maintained at 85° for 24 hours or until all of the starting material had been consumed as evidenced by t.l.c. The reaction was worked-up in the usual manner and the resulting crude product was purified by column
chromatography to afford \( E \) and \( Z-5,6\text{-di-0\text{-acetyl-3-deoxy-1,2\text{-0\text{-isopropylidene-3-C-\{nitro(methoxycarbonyl)methylene\}-\alpha-D-allofuranose (240) as an inseparable mixture in an 88\% yield.}

The n.m.r. of compound 240 exhibited the familiar pattern of low field signals for both \( E \) and \( Z \) isomers. The anomeric protons appeared as over-lapping doublets at \( \delta 5.94 \) and \( \delta 5.91 \) with identical \( J_{1,2} \) values of 5.0 Hz. The H-2 protons appeared as quartets at \( \delta 5.59 \) and \( \delta 5.48 \) with \( J_{2,4} = 2.0 \) Hz and the H-4 protons exhibited quartets at \( \delta 5.84 \) and \( \delta 5.73 \). The elemental analysis of compound 240 also agrees with its proposed structure.

The reduction of the carbon-carbon double bond of compound 240 was accomplished with sodium cyanoborohydride at pH 3-4 to afford the 3-deoxy-3-C-nitro(methoxycarbonyl)-methyl sugar 241 in 97\% yield.

A first-order analysis of the p.m.r. spectrum of 241 (see Figure 9) revealed the following:

1) the multiplicity of resonances showed that, as expected, the compound was a mixture of two components,
2) the anomeric protons appeared as over-lapping doublets at \( \delta 5.87 \) and \( \delta 5.77 \),
3) the branch-chain protons showed as doublets at \( \delta 5.41 \) and \( \delta 5.36 \),
4) the H-3 protons appeared as over-lapping
Figure 9  Partial 100 MHz P.M.R. Spectrum of 5,6-di-O-Acetyl-3-deoxy-1,2-O-iso-
propylidene-3-C-[(R,S)-nitro(methoxycarbonyl)methyl]-α-D-allofuranose (241)
in CDCl₃.
multiplets between δ2.9 and δ3.3, and
5) H-2 and H-4 were shown as over-lapping signals
between δ4.6 and δ5.1.

The details of the location and coupling constants
of the resonances were revealed by a series of irradiation
experiments, the results of which are summarized in Table
6. The irradiations were performed as follows (as usual,
the resonances of the predominant component are labelled
"A" and those the minor component "B")

1) irradiation of the H-1 resonances revealed
H-2 resonances at δ4.97 and δ4.79,
2) irradiation of H-1'A at δ5.36 revealed H-3A
at δ3.01 and irradiation of the doublet at δ4.97
showed that it was proton H-2A with a coupling
constants of J_2,3 = 5.0 Hz and J_1,2 = 3.5 Hz.
The value of J_1',3 = 9.0 Hz was read directly
from the splitting of the H-1'A doublet and
therefore the J_3,4 value was determined to be
9.0 Hz.
3) a first order analysis thus revealed that
proton H-2B at δ4.79 was coupled to H-1B at
5.87 with J_1,2 = 4 Hz and to H-3B (≈δ3.1)
with J_2,3 = 5.5 Hz.
The J_2,3 values of 5.0 and 5.5 Hz for the major
and minor isomers, respectively, indicated an
allo configuration for compound 241. Additional proof of
TABLE 6 Chemical Shifts and Coupling Constants of Protons of 5,6-Di-\(\text{O}\)-acetyl-3-deoxy-1,2-\(\text{O}\)-isopropylidene-3-\(\text{C}\)-[(\(R\),\(S\))-nitro(methoxycarbonyl)methyl]\)-\(\alpha\)-\(\text{D}\)-allofuranose (241).

<table>
<thead>
<tr>
<th>PROTON</th>
<th>(\delta)</th>
<th>COUPLING CONSTANTS (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>major isomer</td>
</tr>
<tr>
<td>H-1</td>
<td>5.77</td>
<td>(J_{1,2} = 3.5)</td>
</tr>
<tr>
<td>H-2</td>
<td>4.97</td>
<td>(J_{1,2} = 3.5)</td>
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<td></td>
<td></td>
<td>(J_{2,3} = 5.0)</td>
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<td>H-3</td>
<td>3.01</td>
<td>(J_{2,3} = 5.0)</td>
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<tr>
<td></td>
<td></td>
<td>(J_{3,4} = 9.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(J_{3,1'} = 9.0)</td>
</tr>
<tr>
<td>H-1'</td>
<td>5.36</td>
<td>(J_{3,1'} = 9.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>minor isomer</td>
</tr>
<tr>
<td>H-1</td>
<td>5.87</td>
<td>(J_{1,2} = 4.0)</td>
</tr>
<tr>
<td>H-2</td>
<td>4.79</td>
<td>(J_{1,2} = 4.0)</td>
</tr>
<tr>
<td></td>
<td></td>
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</tr>
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<tr>
<td>H-1'</td>
<td>5.41</td>
<td>(J_{3,1'} = 10.0)</td>
</tr>
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</table>

its structure was provided by hydrolyzing the 5,6-\(\text{O}\)-isopropylidene blocking group of the 3-deoxynitro ester 236 and immediately acetylating the resulting diol 242 to yield a compound identical to the previously described diacetyl nitro ester 241. An unequivocal proof of structure of compound 236 was provided in section 3.5.2.
3.5.4 Synthesis of 1,2,5,6-Tetra-O-acetyl-3-deoxy-3-C-[(R,S)-nitro(methoxycarbonyl)methyl]-α,β-D-allofuranose (243).

Before a nucleoside synthesis could be attempted, the 1,2-O-isopropylidene blocking group of compound 241 had to be replaced by acetates. Acid catalyzed hydrolysis of the acetal, followed by acetylation was not the method of choice because of the possibility of lactone formation between the branch-chain ester and the C-2 hydroxyl during the hydrolysis. This would present two problems:

1) since compound 141 is a mixture of diastereomeric isomers, differing in configuration at the branch-chain carbon, one isomer might favour lactonization whereas the other might not due to
steric considerations. The probable formations of α and β anomeric products from each of the isomers would thus result in a complex product mixture which could prove difficult to separate and/or purify, and

2) the formation of a lactone would prevent acetylation of the C-2 hydroxy. As previously discussed in the Introduction (section 4.2), the absence of a "participating" group at C-2 favours the production of α and β nucleosides whereas the presence of a 2-0-acetoxy group favours predominant formation of the desired β-nucleoside.

For these reasons the isopropylidene group of compound 241 was removed by acetolysis to afford the tetra-0-acetyl nitro ester 243 directly. Thus, compound 241 was dissolved in an acetic acid solution containing 10 equivalents of acetic anhydride and 0.2 equivalents of p-toluenesulphonic acid. After heating at 80-85° for two hours, the reaction mixture was worked-up to afford compound 243 in 98% yield.

The p.m.r. spectrum of compound 243 was understandably complex. The main features were:

1) the anomeric protons appeared as a doublet at δ6.44 with $J_{1,2} = 4.0$ Hz and a doublet at
δ6.19 with \( J_{1,2} = 2.8 \text{ Hz} \) and on the basis of the coupling constants \(^{213}\) were designated as the H-1 protons of α and β isomers, respectively. The integrated areas of the resonances gave an α/β ratio of 1:4.

2) two ester resonances at δ3.92 and δ3.84 equivalent to 3 protons, and

3) five acetate signals between δ2.22 and δ2.06 equivalent to 12 protons.

Although the p.m.r. spectrum showed that compound 243 was a mixture of α and β anomers, no solvent system could be found to afford separation of the isomers. Nevertheless, the elemental analysis of the mixture was well within acceptable limits. The high resolution mass spectrum of the mixture showed an intense peak corresponding to a mass of 390.1048 and a molecular formula of \( C_{15}H_{20}NO_{11} \).
The calculated mass of the parent ion -OAc was 390.1036.

3.5.5 Attempted Nucleoside Synthesis with Compound 243.

The stannous chloride catalyzed silyl Hilbert-Johnson procedure\textsuperscript{134-140,146} (see Introduction, section 4.2.3.2) was the method chosen for the attempted condensation of the per-acetyl nitro ester 243 with bis(trimethylsilyl)thymine (244)\textsuperscript{191}.

Compounds 243 and 244 (2 equiv.) were dissolved in dichloromethane and 1.4 equivalents of stannous chloride were added. After stirring for 24 hours at room temperature, the solution was diluted with a saturated aqueous solution of sodium bicarbonate and extracted with ethyl acetate. The combined extracts were then dried (\textit{Na}_2\textit{SO}_4) and evaporated to yield an amorphous solid. However, if the solid was carefully washed with chloroform, then evaporation of the filtrate yielded a yellow syrup which proved to be (p.m.r. spectroscopy) the starting sugar 243. The remaining solid revealed itself to be predominantly thymine.

Even when the reaction was repeated using 1,2-dichloroethane as the solvent and the reaction temperature was increased to 63° for periods up to 24 hours, no evidence of nucleoside formation was found.

At this time it was decided to proceed towards the prime objective of our research - the synthesis of a
hexofuranosyl amino acid nucleoside analogue of polyoxin
J - rather than pursue the 3-deoxy analogue sequence any
further.

3.6 Attempted Synthesis of 1-[3'-C-((S)-carbomethoxy(amo
methyl)-β-D-allofuranosyl]thymine.

The synthetic strategy envisioned for the synthesis
of an analogue of the nucleoside moiety of polyoxin J
from the L-amino ester 205 involved:

1) blocking of the amino functionality with a tri-
fluoroacetyl group

2) hydrolysis of the 5,6-0-isopropylidene group with
accompanying acetyl migration

3) acetylation of free hydroxy groups

4) acetolysis of the 1,2-0-isopropylidene group

5) condensation of the per-acetyl sugar with bis-
(trimethylsilyl)thymine, and

6) removal of the acetates.

3.6.1 Synthesis of Methyl L-2-(3-0-acetyl-1,2;5,6-di-
0-isopropylidene-α-D-allofuranos-3-yl)-N-tri-
fluoroacetyl glycinate (245).

Trifluoroacetylation of methyl L-2-(3-0-acetyl-1,
1,2:5,6-di-0-isopropylidene-α-D-allofuranos-3-yl)glycinate
(205) was accomplished by adding trifluoroacetic anhydride
to a cooled (-10°) solution of the sugar in chloroform-
pyridine. After 1/2 hour the reaction was worked-up and the crude product was chromatographed on a column of silica gel to afford compound 245 in an 85% yield.

The p.m.r. spectrum of compound 245 gave indirect evidence for the existence of the N-trifluoroacetate. Only one resonance disappeared upon addition of deuterium oxide to the sample and that was a broad one-proton doublet at δ7.83, typical of N-acetyl amide protons which are generally found between δ6.6 and δ7.0. The corresponding N-acetyl compound 209 exhibited an amide resonance at δ6.68 and the down-field shift to δ7.83 may be attributed to the inductive effect of the trifluoromethyl group. The branch-chain proton H-1' appears as a doublet at δ5.88 with J_{1',NH} = 9.0 Hz. In all the spectrum showed resonances of 26 protons consistent with the proposed structure of compound 245. Confirmation of the structure was provided by the elemental analysis.
3.6.2 Synthesis of Methyl L-2-(3,5,6-tri-0-acetyl-1,2-0-isopropylidene-α-D-allofuranos-3-yl)-N-tri-fluoroacetyl glycinate (246) and Methyl L-2-(5,6-di-0-acetyl-1,2-0-isopropylidene-α-D-allofuranos-3-yl)-N-trifluoroacetyl glycinate (247).

In section 3.4.1 we described the hydrolysis of the 5,6-0-isopropylidene group of compound 209 and the accompanying migration of the 3-0-acetyl group to the C-6 hydroxyl. It was expected that a similar acyl migration would occur during the hydrolysis of compound 245.

With this in mind, the fully blocked amino ester 245 was dissolved in 66% acetic acid and the solution was stirred for 5 hours at 22° and a further 4 h at 40° at which time a t.l.c. of the solution, using 1:1 benzene-ethyl acetate as the developer, showed that the starting material (R_f 0.57) had been consumed to yield a product with an R_f value of 0.30. The product isolated was not characterized but was immediately dried and then acetylated with acetic anhydride-pyridine, for 13 hours, to acetylate primary and secondary hydroxyl groups. T.l.c. of the reaction mixture showed the presence of two products with R_f values of 0.29 and 0.41. The reaction mixture was worked-up and the crude product chromatographed on a column of silica gel to afford a trace amount of the starting material 245 (1%) and the two lower R_f components.
The $R_f$ 0.41 component (29%) possessed a p.m.r. spectrum with the following features: (see Figure 10)
1) the usual resonances of the branch-chain; methyl ester ($\delta 3.78$) and H-1' ($\delta 5.83$) coupled to the broad doublet of the amide proton ($\delta 7.64$) with $J = 10.0$ Hz.
2) three acetate signals, at $\delta 2.10$, $\delta 2.06$ and $\delta 2.03$, equivalent to a total of 9 protons
3) methyl resonances attributed to an isopropy-lidene group, and
4) six resonances consistent with H-1, 2, 4, 5 and 6 of an $\alpha$-allofuranose system.

The lack of evidence of any free hydroxyl groups, the presence of three acetate signals and an elemental analysis and high resolution mass spectrum corresponding to a molecular formula of $C_{20}H_{26}NO_{12}F_3$ indicated that the $R_f$ 0.41 component was the fully blocked tri-0-acetyl sugar 246.

The lower $R_f$ component was isolated as a crystalline solid (65%). Its p.m.r. spectrum (see Figure 11) was similar to that of compound 246 with the following important differences:
1) the presence of only two acetate resonances at $\delta 2.13$ and $\delta 2.06$, and
2) the presence of a sharp singlet at $\delta 3.93$ equivalent to one proton and which exchanged in deuterium oxide.
Figure 10 Partial 100 MHz P.M.R. Spectrum of Methyl 1-2-(3,5,6-tri-O-acetyl-1,2-0-isopropylidene-\(\alpha\)-D-allofuranos-3-yl)-\(\beta\)-trifluoroacetyl glycinate (246) in CDCl\(_3\).
Figure 11 Partial 100 MHz P.M.R. Spectrum of Methyl L-2-(5,6-di-O-acetyl-1,2-0-isopropylidene-α-D-allofuranos-3-yl)-N-trifluoroacetyl glycinate (247) in CDCl₃.
The elemental analysis also agreed with a compound bearing two acetyl groups and one free hydroxy. Reasoning that the acetylation conditions were sufficient to acetylate all primary and secondary hydroxyls, the lower $R_f$ component must therefore be the 3-hydroxy-5,6-di-O-acetyl sugar 247.
It is postulated that the acid hydrolysis of compound 245 yielded the diol 248 which underwent an acyl migration from the C-3 hydroxyl to give the diol 249 to the extent of ~70%. The acetylation of the mixture of compounds 248 and 249 in acetic anhydride-pyridine thus afforded a mixture of the tri-0-acetate 246 and the di-0-acetate 247 in the same proportions as the parent diols.

This hypothesis was supported by an experiment in which compound 247 was recovered unchanged from a solution of acetic anhydride-pyridine after a period of 48 hours. The conversion of 247 into 246 was, however, accomplished using acid catalyzed conditions at elevated temperatures. Similarly, compound 246 was synthesized directly from the hydrolysis mixture by acid catalyzed acetylation at 110° in a 79% yield based on the starting compound 245.

3.6.3 Synthesis of Methyl L-2-(1,2,3,5,6-penta-o-acetyl-α,β-D-allofuranos-3-yl)-N-trifluoroacetyl glycinate (250).

Compound 246 was dissolved in a mixture of 1:1 acetic anhydride-acetic acid and p-toluenesulphonic acid monohydrate and the resulting solution was heated at 110° for two hours until the t.l.c. (6:4 benzene-ethyl acetate) showed that the starting material had been consumed. The
crude product isolated from the reaction mixture was chromatographed to afford the per-acetylated compound 250 in a 47% yield.

From its p.m.r. spectrum (see Figure 12) it was evident that compound 250 was a mixture of $\alpha$ and $\beta$ anomers. The anomeric proton of the minor isomer appeared as a doublet at $\delta 6.42$ coupled to the H-2 doublet at $\delta 5.64$ with $J_{1,2} = 5.1$ Hz. H-1 and H-2 of the major isomer appeared as superimposed signals at $\approx \delta 6.02$. When the spectrum of compound 250 was obtained at 270 MHz, the signals were separated into two singlets at $\delta 6.01$ and $\delta 6.04$ and on this basis the major isomer is postulated to be the $\beta$ isomer while the minor isomer is postulated to be the $\alpha$ isomer.
Figure 12 Partial 100 MHz P.M.R. Spectrum of Methyl \( \text{L-2-}(1,2,3,5,6\text{-penta-0\text{-acetyl-}} \alpha, \beta-\text{D-allofuranos-3-yl})-\text{N-trifluoroacetyl glycinate (250)} \) in CDC\(_3\).
3.6.4 Attempted Nucleoside Syntheses Using Per-acyl Amino Ester 250.

A number of unsuccessful attempts were made to synthesize nucleosides from compound 250 using the stannous chloride catalyzed silyl Hilbert-Johnson and fusion methods (see Introduction, sections 4.2.1 and 4.2.3). Although the primary objective was the synthesis of a thymine nucleoside to serve as an analogue of polyoxin J, some attempts were also made to synthesize purine nucleosides of compound 250.

3.6.4.1 Stannous Chloride Catalyzed Silyl Hilbert-Johnson Procedure.

The reaction of compound 250 with \( \text{N}^6 \)-benzoyl-\( \text{N}^6,9 \)-bis-(trimethylsilyl) adenine (114) in 1,2-dichloroethane at 70° and in the presence of 1.4 equivalents of stannous chloride produced no discernable nucleoside products. The t.l.c. of the product isolated after work-up of the reaction showed the presence of only the starting material 250 and \( \text{N} \)-benzoyl adenine and following removal of the purine, compound 250 was recovered in an 86% yield.

Similar results were obtained when bis(trimethylsilyl)thymine 244 was used as the base and even the use of more polar acetonitrile as the solvent produced no discernable reaction.
3.6.4.2 Fusion Procedures. Synthesis of 1,1'-Anhydro-
2,3,5,6-tetra-O-acetyl-3-C-(R)-methoxycarbonyl-
1(R),1'(S)-N-trifluoroacetoepimo-\alpha-Dallo-
furanose (252).

The fusion of compound 250 with \( \text{N-benzoyl adenine,} \)
2,6-dichloropurine or silylated \( \text{N-benzoyl adenine 114,} \)
with or without acid catalyst, produced no nucleoside
products.

It was therefore decided to convert compound 250 into its more reactive bromo sugar derivative 251 prior
to the fusion reaction. Compound 250 was dissolved in
dichloromethane, the solution was cooled to 0°, and a
slow stream of anhydrous hydrogen bromide was bubbled
through the solution for 2 1/2 hours. The residual acidic
components were then removed by distillation under reduced
pressure and the resulting bromo-sugar 251 was intimately
mixed with 1.8 equivalents of silylated \( \text{N-benzoyl adenine 114.} \)
This mixture was fused for 20 minutes at 160°/
15 torr to yield, after work-up, a crude product that was
shown by t.l.c. (6:4 benzene-ethyl acetate) to be composed
of two components. Column chromatography of the crude
product afforded a high \( R_f \) component (37%) which was shown
to be the starting material 250 and a low \( R_f \) component
252 (52%, based on starting material consumed).

T.l.c. had already shown that compound 252 was
a charring, non-U.V. active compound and therefore probably
a carbohydrate derivative rather than a nucleoside. The p.m.r. spectrum (see Figure 13) of 252 confirmed this assumption as there were none of the low field resonances present that would be expected for an N-benzoyl adenine nucleoside. Several of the easily recognizable signals included:

1) four acetate methyl peaks at δ2.10, 2.06, 2.01 and 2.00
2) a methyl ester signal at δ3.76, and
3) resonances attributable to H-4, 5 and 6 of a hexofuranose system. The H-4 doublet at δ5.01 was coupled to the H-5 sextet at δ5.30 which was in turn coupled to two H-6 quartets at δ4.44 and 4.01.

This left three unaccounted-for resonances, each equivalent to one proton. The first appeared as a broadened triplet at δ5.59, the second as a broad doublet or overlapping doublets at δ5.39 and the third as a broad singlet at δ4.76. Noticeably absent was a resonance attributable to an amide proton or any other exchangeable proton, since the addition of deuterium oxide to the n.m.r. sample did not change its p.m.r. spectrum. It was therefore assumed that the proton of the branch-chain nitrogen had been substituted by an acyl or alkyl group to afford a tertiary amine.
Figure 13  Partial 100 MHz P.M.R. Spectrum of 1,1'-Anhydro-2,3,5,6-tetra-O-acetyl-3-C-(R)-methoxycarbonylmethyl-1(R),1'(S)-N-trifluoroacetoepimo-β-D-allofuranose (252) in CDCl₃.
The first clue to the identity of compound 252 was the line broadening and apparent multiplicity of the three unassigned resonances. In 1963, Hanessian and Haskell reported the synthesis and p.m.r. spectrum of 5-acetamido-5-deoxy-5-xylofuranose 253. They explained the existence of two acetyl methyl peaks and two anomeric doublets by postulating that 253 was a mixture of α and β anomers. A similar multiplicity was shown to exist in the p.m.r. spectrum of methyl 4-acetamido-4-deoxy-L-erythrofuranoside 254 with the appearance of two anomeric doublets (δ4.95 and δ5.04), two methoxyl singlets (δ3.36 and δ3.41) and two acetyl methyl singlets (δ2.08 and δ2.12). In both cases it was later shown that such multiplicity was not due to a mixture of anomeric compounds, but instead to the existence of two rotational isomers resulting from restricted rotation about the CO-N bond arising from resonance conjugation between the p-orbital of the nitrogen and the p-orbital of the π-electron system. In the case of compound 254 such a conjugation results in the dipolar structures 254a and 254b.

With this in mind the p.m.r. spectrum of compound 252 was readily interpreted as that of the two rotational isomers of 1,1'-anhydro-2,3,5,6-tetra-O-acetyl-3-C-(R)-methoxycarbonylmethyl-1(R),1'(S)-N-trifluoroacetooepimino-β-D-allofuranose. The low field resonance at δ5.59 was thus seen as the two overlapping doublets of the anomeric
proton which are coupled to the overlapping doublets of H-2 at δ5.39 with J₁,₂ = 1.5 Hz. The branch-chain protons thus appeared as a broadened singlet at δ4.76.

The high resolution mass spectrum of compound 252 supported the proposed structure with the appearance of a peak corresponding to a mass of 514.1194. The calculated mass of a C₁₉H₂₃NO₁₂F₃ (M⁺ + 1) ion was 514.1173. The elemental analysis of compound 252 also agreed with its proposed structure.

The mechanism of the reaction is probably very similar to those proposed for the formation of nucleosides (see Introduction, section 4.2) with the ionic intermediates
and 256 being formed prior to the intramolecular N-glycosidation reaction which afforded compound 252.


A successful nucleoside synthesis was finally accomplished by application of the recently developed\textsuperscript{150, 152, 153} variation of the silyl Hilbert-Johnson procedure in which silver trifluoromethylsulfonate (silver triflate) is used as the catalyst.

Compound 250 was dissolved in anhydrous dichloromethane and the solution was cooled to 0° prior to introduction of a slow stream of anhydrous hydrogen bromide. After 2 hours the solvents and residual acid were removed by vacuum distillation. The product was redissolved in dichloromethane and then slowly added to a mixture of silver triflate and dichloromethane which was maintained at -70°. Five minutes after the addition was complete, a solution of bis(trimethylsilyl)thymine (244) was slowly added and the resulting reaction mixture was maintained at -70° for an additional 2 hours before allowing it to spontaneously reach room temperature over the next 18 hours. The crude product obtained from the work-up of the reaction was chromatographed on a column of silica gel, using 10:5:1 benzene-ether-ethanol as the developer,
to afford the starting material 250 (54%) and the thymine nucleoside 257 (93% based on starting material consumed).

The high resolution mass spectrum of nucleoside 257 showed a parent peak at 639.1530 mass units (theoretical value 639.1515) and a fragment at 514.1140 corresponding to loss of the base from the parent ion (theoretical value 514.1173).

The p.m.r. spectrum of compound 257 (see Figure 14) possessed all the characteristics expected. The presence of a thymine base was shown by a methyl singlet at δ1.93, a vinyl proton singlet at δ7.24 and an amide singlet at δ9.55. The elements of the branch-chain were exhibited in the methyl ester peak at δ3.84 and the mutually coupled amide and branch-chain proton doublets at δ7.63 and δ5.61, respectively. The resonances of the six remaining protons of the sugar skeleton and the four acetates were also readily identifiable. One unexpected feature was the large coupling constant of 7.2 Hz between H-1' and H-2'. It is well established that the anomeric configuration of aldofuranosyl derivatives cannot be determined from the J1',2' coupling constants of a single isomer and therefore the designation of compound 257 as a β-nucleoside was based on mechanistic considerations.

Compared with the stannous chloride method 134-140, 146, the perfluoroalkane sulphonate or perchlorate
Figure 14  Partial 100 MHz P.M.R. Spectrum of \(1-[2',3',5',6'-\text{Tetra-}O\text{-acetyl-}3'-\text{C-}(\text{S})-N\text{-trifluoroacetyl-carbomethoxy(}\text{amino})\text{methyl})-\beta-\text{D-allofuranosyl]}\text{thymine} (257)\) in CDCl\(_3\).
catalyzed reaction of silylated pyrimidines, with sugars possessing 2-α-acyloxy groups, shows the same high degree of stereoselectivity favouring the exclusive formation of γ-N-1-nucleosides from 2-alkyl-1,2-dioxolenium cation intermediates (see Introduction, section 4.2).

Indirect spectroscopic evidence for the existence of such an intermediate as the predominant alkylating agent...
has been provided by p.m.r. observations\textsuperscript{152} of 2,3,5-tri-\textsubscript{0}-acetyl-D-ribofuranosyl triflate \textsuperscript{258}. The formation of the thymine $\beta$-nucleoside \textsuperscript{257} is therefore consistent with the cation \textsuperscript{255} acting as a stereospecific alkylating agent. Further support for the assigned $\beta$-configuration was provided by the c.d. spectrum of compound \textsuperscript{257} which exhibited a positive Cotton effect at 268 nm.\textsuperscript{226}

3.6.6 Attempted Unblocking of 1-[2',3',5',6'-Tetra-\textsubscript{0}-acetyl-3'-C-(S)-N-trifluoroacetyl-carbomethoxy-(amino)methyl-$\beta$-D-allofuranosyl]thymine (257).

Several methods were employed in an attempt to hydrolyse the acetate and ester blocking groups of the fully blocked nucleoside \textsuperscript{257}.

Deacetylation of the \textsubscript{0}-acetyl blocking groups using sodium methoxide in methanol\textsuperscript{223} was the initial unblocking procedure employed. The nucleoside \textsuperscript{257} was dissolved in anhydrous methanol containing a catalytic amount of sodium methoxide and after several hours t.l.c. showed a large proportion of the starting material still remained. After 24 hours, t.l.c. showed that all of the nucleoside \textsuperscript{257} had been consumed and the reaction mixture was deionized in the usual manner using IRC-50 (H\textsuperscript{+}) cation exchange resin. The amorphous solid isolated from the work-up of the reaction was then dissolved in deuterium oxide and a p.m.r. spectrum of the solution was obtained. It was immediately obvious that the deacetylation reaction had not proceeded to completion as the region of the p.m.r.
spectrum around $\delta 2$ contained a great many acetate methyl resonances of widely varying intensities. The low field region of the spectrum, where the anomeric proton resonances are usually observed, was similarly complex indicating that the crude product was in fact a mixture of a great many compounds. When the procedure was repeated and the reaction mixture was left at room temperature for 7 days, the p.m.r. spectrum of the product obtained from the reaction was as complex as that obtained previously.

The complex composition of the product mixture was confirmed by paper chromatography using 10:4:3 ethyl acetate-pyridine-water as the solvent. Six components were detected on the paper chromatogram with $R_f$ values of 0.11, 0.15, 0.34, 0.65, 0.73, and 0.81. All of the zones were visible under U.V. light with the exception of the $R_f$ 0.81 component, while the $R_f$ 0.11, 0.15, 0.34 and 0.81 components gave a positive reaction with ninhydrin. The $R_f$ 0.34 zone was by far the predominant component of the product mixture and was therefore isolated from a preparative scale chromatogram by eluting the zone with water. Unfortunately, the p.m.r. spectrum of this component was as complex as that of the original product mixture.

Column chromatography of reaction mixture on a column of Bio-Rex 70 ($H^+$) resin also failed to effect the separation of an individual identifiable compound.
The second method used for the attempted deacetylation of compound 257 employed methanolic ammonia according to the procedures outlined by Wolfrom and co-workers for the unblocking of O-acetyl/N-trifluoroacetyl nucleosides. According to this procedure the fully blocked nucleoside was dissolved in anhydrous methanol, the solution was saturated with ammonia at 0°C and then kept at room temperature for 5 days. Evaporation of the solvent yielded a product whose p.m.r. spectrum indicated that incomplete deacetylation had once again been achieved. A similarly complex product mixture was obtained after a prolonged ammonolysis attempt of 14 days duration. Once again, preparative paper chromatography failed to achieve the isolation of any single identifiable component.

The third method used for the deacetylation of the nucleoside 257 involved saturating a cooled (0°C) methanolic solution of 257 with hydrogen chloride and keeping the resulting solution at room temperature for 18 hours according to the procedure outlined by Wolfrom and co-workers. Once again, a complex mixture of partially de-acetylated compounds was isolated.

Finally, the triethylamine-methanol and triethylamine-methanol-water methods previously employed for the deacetylation of O-acetyl nucleosides and polysaccharides failed, in our hands, to completely remove all of the O-acetates of compound 257.
Although the failure to unblock the per-acetyl nucleoside 257 represents a synthetic impasse, in several respects the condensation of methyl nitroacetate with 1,2;5,6-di-\text{-}\text{O}-\text{isopropylidene-}\alpha\text{-}\text{D-ribo-hexofuranos-3-ulse} (25) as a method for producing analogues of the polyoxin complex must still be considered a success. Firstly, it has been shown (see Section 3.3.3) that the reaction afforded a glycos-3-yl \(\alpha\)-amino acid, in good yield, that possessed the same absolute configuration as the naturally occurring compounds and secondly, the reaction provided a route to the analogous pentofuranosyl derivatives (see Section 3.4.3). Finally, the application of the triflate catalyzed nucleoside synthesis proved successful despite the steric hinderance offered by the branched-chain allofuranose (see Section 3.6.5). Hopefully, with the wide variety of alternative ester and ether blocking groups available for the protection of alcohols, the synthesis of a free peptidyl nucleoside will be attained using this procedure.
IV. EXPERIMENTAL

1. General Methods

Mass spectra were recorded on an A.E.I. MS9 spectrometer. Optical rotations were measured with a Perkin Elmer model 137 spectrometer and circular dichroism (c.d.) measurements were performed on a JASCO ORD/UV-5 spectropolarimeter or a JASCO J-20 Automatic Recording Spectropolarimeter. Ultraviolet spectra (u.v.) were recorded on a Carey 15 spectrometer. Melting points were determined on a Leitz Microscope heating stage model 350 and are corrected. Infrared spectra (i.r.) were recorded on a Perkin Elmer model 727B spectrometer. Elemental analyses were performed by Mr. P. Borda of the Department of Chemistry, University of British Columbia.

1.1 Chromatography

1.1.1 Column Chromatography

Column chromatography was performed using silica gel indicated as "silica gel H for t.l.c. acc. to Stahl (Type 60)" (E.M. Reagents). Columns were pressurized above the solvent reservoir to a pressure of 7-12 psi.
1.1.2 **Thin Layer Chromatography**

All thin layer chromatography was performed using silica gel for t.l.c. (Camag), containing 5% CaSO₄ and u.v. indicator. Compounds were detected by ultraviolet absorption, by spraying with 50% sulphuric acid followed by heating on a hot plate, by spraying with a 0.3% solution of ninhydrin in n-butanol followed by warming at 110° in an oven and/or by spraying with a dilute solution of potassium permanganate.

1.1.3 **Paper Chromatography**

Paper chromatograms were performed using Whatman No. 1 paper. Nucleosides were detected with ultraviolet light.

1.2 **Nuclear Magnetic Resonance Spectroscopy**

Proton magnetic resonance spectra (p.m.r.) were recorded on Varian T-60, HA-100, XL-100 or Bruker 270 MHz spectrometers. Absorptions are given in δ units with tetramethylsilane (unless otherwise stated) as the internal standard (set at δ = 0). The following abbreviations are used in describing p.m.r. spectra: s = singlet, d = doublet, t = triplet, q = quartet, qn = quintet, sex = sextet, sept = septet, oct = octet, and m = multiplet. Chemical shifts (δ values) were measured at the mid-point of the absorptions and are not corrected. P.m.r. spectra were initially subjected to a first order analysis in order to arrive at
an internally consistent set of coupling constants (J values) and, wherever possible, the assignments thus arrived at were confirmed by irradiation experiments.
2. **Synthesis of Branched-Chain Amino Sugars.**

Treatment of 1,2:5,6-Di-O-isopropylidene-α-D-ribo-hexofuran-3-ulose (25) with Sodium Cyanide and Methyl Nitroacetate.

Procedure A to Yield Predominantly 3-C-Cyano-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (26).

To a solution of ketose 25 (1.43 g) in anhydrous ethanol (2 ml) was added sodium cyanide (0.290 g). A solution of methyl nitroacetate (0.706 g) in anhydrous ethanol (2 ml) was added dropwise and the reaction mixture was stirred for 18 h at room temperature. The ethanol was removed by evaporation under reduced pressure. After the addition of 10 ml of chloroform, a precipitate of non-carbohydrate material was removed by filtration. Water (10 ml) was added and the mixture extracted with chloroform (5 x 20 ml). The combined chloroform extracts were dried over magnesium sulphate, filtered, and evaporated to yield a syrup (1.13 g). The crude product was chromatographed on a column of silica gel (50 g, 24 x 2.8 cm) using 1:1 benzene-ethyl acetate as developer to yield ketose 25 (0.44 g) and a syrup (0.739 g, 61%) which crystallized upon standing. Compound 26 was recrystallized from benzene-hexane; m.p. 99-100°, [α]$_D^{24}$ + 51.6° (c 1.4, chloroform); n.m.r. (CDCl$_3$): δ 5.92 (d, 1, J$_{1,2}$ 4 Hz, H-1), 4.55 (d, 1, H-2), 4.4-4.0 (m, 5, one proton exchanges in D$_2$O), 1.55, 1.51, 1.36 and 1.34 (4s, 12, CH$_3$).
Anal. Calc. for $C_{13}H_{19}NO_6$: C, 54.73; H, 6.71; N, 4.91.

Found: C, 54.87; H, 6.77; N, 4.92.

The n.m.r. spectrum of the syrup showed the presence of both gluco-cyanohydrin 26 and allo-cyanohydrin 27 in a ratio of 13:1.

Procedure B to Yield Predominantly $3\text{-C-Cyano-1,2:5,6-di-O-isopropylidene-\ensuremath{\alpha}-D-allofuranose}$ (27).

To a mixture of sodium cyanide (0.219 g) in anhydrous ethanol (2 ml) was added methyl nitroacetate (0.525 g), followed immediately by a solution of ketose 25 (1.14 g) in ethanol (5 ml). After the reaction mixture was stirred for 18 h at room temperature, the precipitate of non-carbohydrate material was removed by filtration. Evaporation of the filtrate afforded a syrup which was triturated with water (20 ml) and extracted with chloroform (5 x 20 ml). The combined chloroform extracts were dried (MgSO$_4$), filtered, and evaporated to yield a clear syrup. This syrup was chromatographed on a column of silica gel (130 g, 5 x 16 cm) using 4:6 benzene-ethyl acetate as developer to yield ketose 25 (0.247 g) and a syrup (0.986 g). The syrup was crystallized from benzene-hexane to afford the title com-
pound 27 (0.75 g, 85% based on reacted ketose); m.p. 61-64°, 
[a]^{24}_{D} + 8.6° (c 1.4, chloroform); n.m.r. (CDCl₃): δ 5.94
(d, 1, J₁,₂ 4 Hz, H-1), 4.92 (d, 1, H-2), 4.72 - 3.83 (over-
lapping signals, 4, H-4,5 and 6), 3.77 (s, 1, exchanges in
D₂O, OH), 1.61, 1.48 and 1.40 (3s, 12, CH₃).

Anal. Calc. for C₁₃H₁₉NO₆: C, 54.73; H, 6.71; N, 4.91.
Found: C, 54.89; H, 6.81; N, 4.98.

The n.m.r. spectrum of the syrup obtained from the
mother liquor after the crystallization of 27 showed the
presence of 27, the gluco-cyanohydrin 26, and the methyl
nitro acetate addition compound 162. The ratio of products
27, 26, and 162 was 87:6:7, respectively. Attempts to
obtain pure 162 by repeated column chromatography led to
its decomposition.

Procedure C

The reaction was carried out in the same way as de-
scribed in Procedure B up to the chromatographic separation.
The product mixture (1.266 g) was acetylated with acetic
anhydride (10 ml) and p-toluenesulfonic acid monohydrate
(0.17 g) for 5 h at 80-85°. The volatile components of
the mixture were removed by co-distillation with toluene
under reduced pressure to yield a dark syrup which was dis-
solved in chloroform (100 ml) and then washed with water.
(3 x 15 ml). The chloroform solution was dried over sodium sulphate and then evaporated to afford a syrup which was chromatographed on a column of silica gel (110 g, 3 x 40 cm) using 9:1 benzene-ethyl acetate as developer to afford the acetates of the gluco-cyanohydrin 163 (9 mg, 0.8%), the allo-cyanohydrin 164 (188 mg, 15.6%), the methyl nitroacetate addition compound 165 (40 mg, 2.6%), and the tri-0-acetyl allo-cyanohydrin 166 (651 mg, 47.6%). The ratio of yields of compounds 163, 164, 165 and 166 was 23:1:4:72, respectively.

3-0-Acetyl-3-C-cyano-1,2:5,6-di-0-isopropylidene-a-D-glucofuranose (163).

The gluco-cyanohydrin 26 (400 mg) was acetylated with acetic anhydride (4 ml) and p-toluenesulfonic monohydrate (20 mg) for 0.5 h at 80°. The product was worked-up in the usual way to afford 490 mg of a syrup which was chromatographed on silica gel (40 g, 2 x 33 cm) using 8:2 benzene-ethyl acetate as the developer. The gluco-acetate 163 (381 mg, 83%) was crystallized from hexane; m.p. 79-80°, $[\alpha]_D^{23} + 45^o$ (c 1.3, chloroform); n.m.r. (CDCl$_3$): 6 6.00 (d, 1, J$_{1,2}$ 4 Hz, H-1), 4.48-4.00 (overlapping signals, 4, H-4, 5, and 6), 2.13 (s, 3, OAc), 1.63, 1.57, 1.43, and 1.38 (4s, 12, CH$_3$).

Anal. Calc. for C$_{15}$H$_{21}$NO$_7$: C, 55.04; H, 6.47; N, 4.28. Found: C, 55.22; N, 6.56; N, 4.29.
3-0-Acetyl-3-C-cyano-1,2:5,6-di-0-isopropylidene-\(\alpha\)-D-allo-
\(\text{furanose (164).}

The \(\text{allo-cyanohydrin 27 was acetylated and purified}
using the same procedure utilized for the preparation of
compound 163. Compound 164 was crystallized from hexane;
m.p. 115-115.5°, \([\alpha]_D^{21} + 38° (c 1.1, \text{chloroform}); n.m.r.
(CDC\(\text{1}_3\)): \(\delta 5.95 \text{ (d, 1, } J_{1,2} 4 \text{ Hz, H-1), 5.21 \text{ (d, 1, H-2),}
4.52-4.32 \text{ (oct, 1), 4.25-4.00 (overlapping signals, 3),}
2.14 \text{ (s, 3, OAc), 1.50, 1.45, 1.36, and 1.32 (4s, 12, CH}_3\).}

\text{Anal. Calc. for C}_{15}\text{H}_{21}\text{NO}_7: C, 55.04; H, 6.47; N, 4.28.
Found: C, 55.13; H, 6.45; N, 4.40

3,5,6-Tri-0-acetyl-3-C-cyano-1,2-0-isopropylidene-\(\alpha\)-D-
\(\text{allofuranose (166).}

The \(\text{allo-cyanoacetate 164 (200 mg) was treated with}
66\% acetic acid for 24 h at room temperature. The acetic
acid was then removed by co-distillation with toluene under
reduced pressure. The residual syrup was then acetylated
in the usual way using acetic anhydride and pyridine to
afford the tri-0-acetate 166 which was purified by column
chromatography on silica gel (30 gm, 2.3 x 19.5 cm) using
8:2 benzene-ethyl acetate as developer. Compound 166 (227 mg,
92.5\%) was crystallized from benzene-hexane; m.p. 131.5-
132.5°, \([\alpha]_D^{26} + 73° (c 1.0, \text{chloroform); n.m.r. (CDC\(\text{1}_3\)):}
\(\delta 5.99 \text{ (d, 1, } J_{1,2} 4 \text{ Hz, H-1), 5.44 \text{ (oct, 1, } J_{4,5} 7.5 \text{ Hz,}
J_{5,6b} 5 \text{ Hz, } J_{5,6a} 2.8 \text{ Hz, H-5), 5.26 \text{ (d, 1, H-2), 4.62 (q,}
\(\ldots\)}
1, J_{6a,6b} 12.5 Hz, H-6a), 4.36 (d, 1, H-4), 4.23 (q, 1, H-6b), 2.18, 2.10, and 2.08 (3s, 9, OAc), 1.55 and 1.36 (2s, 6, CH\textsubscript{3}).

Anal. Calc. for C\textsubscript{16}H\textsubscript{21}NO\textsubscript{9}: C, 51.75; H, 5.70; N, 3.77. Found: C, 51.61; H, 5.60; N, 3.55.

3-C-Acetamidomethyl-1,2:5,6-di-0-isopropylidene-\textalpha-D-gluco-furanose (54) and 3-C-Acetamidomethyl-1,2:5,6-di-0-isopropylidene-\textalpha-D-allofuranose (53).

The gluco-cyanohydrin 26 (100 mg) was dissolved in tetrahydrofuran (THF) (1 ml) and added dropwise to a mixture of lithium aluminum hydride (LAH) (50 mg) and THF (4 ml). After stirring for 1 1/2 h, the excess LAH was decomposed by the addition of ethyl acetate (3 ml). The solution was then filtered, evaporated, and dissolved in chloroform (20 ml). The chloroform was washed with water (2 x 5 ml), dried over sodium sulphate, and evaporated to give a crystalline white solid in quantitative yield; m.p. 112-113.5\degree.

This solid (100 mg), without further purification, was dissolved in anhydrous methanol (5 ml) and acetic anhydride (1 ml). After 24 h, the mixture was poured into ice water (30 ml) and the solution was extracted with chloroform (5 x 20 ml). The combined extracts were dried over sodium sulphate and evaporated to yield 90 mg (79%) of a clear
colourless syrup. Crystallization of compound 54 from benzene-ether gave white crystalline needles (76 mg, 66%); m.p. 126-127°, $[\alpha]_{D}^{24} + 67.3^\circ$ (c 1.46, ethanol); (Lit. values$^98$; m.p. 120-121°, $[\alpha]_{D}^{23} + 63.8$ (c 1.44, ethanol)); n.m.r. (CDCl$_3$): $6.56$ (br t, 1, J$_{1'a}$,NH 5 Hz, J$_{1'b}$,NH 7 Hz, exchanges in D$_2$O, NHAc), 5.81 (d, 1, J$_{1,2}$ 4.5 Hz, H-1), 4.90 (s, 1, exchanges in D$_2$O, OH), 5.67 (sex, 1, J$_{4,5}$ 8.0 Hz, J$_{5,6a}$ 6.0 Hz, J$_{5,6b}$ 5.5 Hz, H-5), 4.31 (d, 1, H-2), 4.09 (q, 1, J$_{6a,6b}$ 8.5 Hz, H-6a), 3.95 (q, 1, H-6b), 3.75 (d, 1, H-4), 3.74 (q, 1, J$_{1'a,1'b}$ 14.5 Hz, H-1'a), 3.46 (q, 1, H-1'b), 2.00 (s, 3, NAc), 1.47, 1.39, 1.33, and 1.28 (4s, 12, CH$_3$).

Compound 27 was treated identically to yield compound 53 (86 mg, 74%) which, after crystallization from benzene-ether-hexane, gave white crystalline needles; m.p. 158-158.5°, $[\alpha]_{D}^{24} + 64.3$ (c 1.43, ethanol); (Lit. value$^98$; m.p. 156-156.5°, $[\alpha]_{D}^{23} + 56.3$ (c 1.0, ethanol)); n.m.r. (CDCl$_3$): $6.27$ (br q, 1, exchanges in D$_2$O, NHAc), 5.77 (d, 1, J$_{1,2}$ 4.0, H-1), 4.23 (d, 1, H-2), 4.17-3.69 (overlapping signals, 4, H-4, 5, and 6), 3.68 (d, 1, J$_{1'a,1'b}$ 14 Hz, J$_{1'a}$,NH 8.0 Hz, H-1'a), 3.29 (q, 1, J$_{1'b}$,NH 3.5 Hz, H-1'b), 3.09 (s, 1, exchanges in D$_2$O, OH), 2.01 (s, 3, NAc), 1.56, 1.43, and 1.33 (3s, 12, CH$_3$).

3-\(\text{C-}\) (Carbomethoxymethyl)-3-deoxy-1,2-\(\beta\)-isopropylidene-\(\alpha\)-D-allofuranose (170).

3-\(\text{C-}\) (Carbomethoxymethyl)-3-deoxy-1,2:5,6-di-\(\beta\)-isopropylidene-\(\alpha\)-D-allofuranose (35) was dissolved in 66\% acetic acid (370 ml) and left for 9 1/2 h at room temperature. The solution was evaporated in vacuo and the remaining traces of acetic acid were removed by repeated co-distillation with toluene at 19 torr to yield a white crystalline solid (13.5 g, 100\%). An analytical sample of compound 170 was prepared by recrystallization from ethanol-ether; m.p. 90-91°, \([\alpha]_{D}^{25} + 70.0° \) (c 0.6, chloroform); n.m.r. (CDCl\(_3\)):

\(\delta\) 5.82 (d, 1, \(J_{1,2} 4\text{Hz}, H-1\)), 4.82 (t, 1, \(J_{2,3} 4\text{Hz}, H-2\)), 3.42 and 3.40 (2s, 7, H-4, 5, 6 and \(\text{CO}_2\text{CH}_3\)), 2.9 - 2.2 (overlapping signals, 3, H-3 and 1'), 2.57 (s, 2, exchanges in \(\text{D}_2\text{O}, \text{OH}\)), 1.82 and 1.65 (2s, 6, \(\text{CH}_3\)).


3-\(\text{C-}\) (Carbomethoxymethyl)-3-deoxy-1,2-\(\beta\)-isopropylidene-\(\alpha\)-D-ribofuranose (171).

To a solution of the diol 170 (13.0 g) in ethanol (200 ml) was added a saturated sodium bicarbonate solution (15 ml) and a solution of sodium periodate (10.7 g, 1 equiv.) in 200 ml water. After stirring for 1 h, a few drops of
ethylene glycol were added to consume any unreacted periodate. Sodium borohydride (0.95 g) was added and the solution was stirred for an additional 1 h. After the addition of acetone (1 ml) to consume any unreacted sodium borohydride, the solution was filtered and extracted with methylene chloride (8 x 100 ml). The combined extracts were dried over sodium sulphate and evaporated to yield a pale yellow syrup. The crude product was distilled at 140° and 0.2 torr to yield compound 171 as an analytically pure syrup (10.3 g, 84%); $[^\alpha]_D^{22} + 64.2^\circ$ (c 2.5, chloroform); n.m.r. (CDCl$_3$): $\delta$ 5.85 (d, 1, $J_{1,2}$ 4 Hz, H-1), 4.80 (t, 1, $J_{2,3}$ 4 Hz, H-2), 4.2 - 3.6 (overlapping signals, 3, H-4, 5), 3.73 (s, 3, CO$_2$CH$_3$), 2.7 - 2.3 (overlapping signals, 3, H-3, 1'), 2.1 (br s, 1, exchanges in D$_2$O, OH), 1.85 and 1.68 (2s, 6, CH$_3$).


5-O-Benzoyl-3-C- (carbomethoxymethyl)-3-deoxy-1,2-O-isopropylidene-$\alpha$-D-ribofuranose (172).

To a solution of compound 171 (10.3 g) in anhydrous benzene (200 ml) was added a solution of benzoyl chloride (5.35 ml) and pyridine (6.8 ml). After 18 h at room temperature, the reaction mixture was passed through a column of grade II alumina and eluted with benzene (100 ml). The combined eluents were evaporated to yield a clear yellow syrup (13.9 g, 95%). Compound 172 was recrystallized from
ether-pet. ether (30-60); m.p. 85-86°, [\(\alpha\)]\textsubscript{D}\textsuperscript{25} = 54.3° (c 2.2, chloroform); n.m.r. (CDCl\textsubscript{3}): \(\delta\) 8.2 - 7.2 (m, 5, COC\textsubscript{6}H\textsubscript{5}), 5.88 (d, 1, \(J\textsubscript{1,2} = 4\) Hz, H-1), 4.83 (t, 1, \(J\textsubscript{2,3} = 4\) Hz, H-2), 4.59 (q, \(J\textsubscript{5a,5b} = 12\) Hz, \(J\textsubscript{5a,4} = 3\) Hz, H-5a), 4.37 (q, 1, \(J\textsubscript{5d,4} = 4.5\) Hz, H-5b), 4.16 (m, 1, H-4), 3.68 (s, 3, CO\textsubscript{2}CH\textsubscript{3}), 2.9 - 2.2 (m, 3, H-3, 1'), 1.50 and 1.31 (2s, 6, CH\textsubscript{3}).

Anal. Calc. for C\textsubscript{18}H\textsubscript{22}O\textsubscript{7}: C, 61.70; H, 6.33. Found: C, 61.70; H, 6.22.

1-0-Acetyl-5-0-benzoyl-3-C-carboxymethyl-3-deoxy-\(\beta\)-D-ribofuranose-2,3-\(\gamma\)-lactone (169).

Compound 172 (13.9 g) was dissolved in 90% (v/v) tri-fluoroacetic acid (130 ml) for 1 1/2 h at room temperature. The solution was evaporated to dryness and the residual traces of acid were removed by repeated co-distillation with toluene under reduced pressure to yield 5-0-benzoyl-3-C-carboxymethyl-3-deoxy-\(\beta\)-D-ribofuranose-2,3-\(\gamma\)-lactone (173) as a white solid (10.6 g, 96.5%). Compound 173 was dissolved in acetic anhydride (45 ml) and pyridine (45 ml) and stirred for 18 h at room temperature. The reaction was poured into ice-water (100 ml) to precipitate the title compound as a white solid (10.1 g, 83%). Compound 169 was recrystallized from iso-propanol-ethyl acetate; m.p. 136-137°, (lit. value 137°); n.m.r. (CDCl\textsubscript{3}): \(\delta\) 8.1 - 7.3 (m, 5, COC\textsubscript{6}H\textsubscript{5}),
6.34 (s, 1, H-1), 4.95 (d, 1, \(J_{2,3} = 6.2\) Hz, H-2), 4.40 and 4.39 (2s, 3, H-4, 5), 3.19 (m, 1, \(J_{3,1'a} = 8.5\) Hz, \(J_{3,1'b} = 2.3\) Hz, H-3), 2.89 (q, 1, \(J_{1'a,1'b} = 17.5\) Hz, H-1'a), 2.54 (q, 1, H-1'b), 1.98 (s, 3, OAc).

2,6-Dichloro-9-[3'-\(\text{C-}(\text{carboxymethyl}-2',3'-\text{\(\gamma\)-lactone})-3'-deoxy-\(\beta\)-\(\text{D-}\)ribofuranosyl] purine (174) and 2,6-Dichloro-9-[3'-\(\text{C-}(\text{carboxymethyl}-2',3'-\text{\(\gamma\)-lactone})-3'-deoxy-\(\alpha\)-\(\text{D-}\)ribofuranosyl] purine (175).

Compound 169 (1.04 g) and 2,6-dichloropurine (0.92 g, 1.5 equiv.) were intimately mixed, dried, and then quickly heated to 160° at a pressure of 20 torr for 5 min. The temperature was allowed to drop to 140° over 2 min at which time the pressure was reduced to 0.2 torr. After 25 min the reaction was cooled to 100°, the vacuum was relieved and ethyl acetate (3 ml) was added. When the melt was completely dissolved, the solution was cooled to 0° and the resulting tan precipitate was removed by filtration. The filtrate was evaporated to yield a foamy white solid. Column chromatography of the crude product on silica gel (150 g, 5 x 17.5 cm) using 1:1 benzene-ethyl acetate as the developer afforded compound 169 (79 mg), \(\beta\)-nucleoside 174 (547 mg, 41%) and \(\alpha\)-nucleoside 175 (648 mg, 48%).

Compound 175 was recrystallized from chloroform to yield a white crystalline solid; m.p. 211-214°, [\(\alpha\)]\text{D}^{25} = -102.5 (c 1.0, chloroform); \(\lambda_{\text{max}}^{\text{MeOH}} = 215\) nm (\(\varepsilon = 21,800\),
and 273 nm (shoulder at \( \nu 230 \text{ nm} \)) (\( \epsilon \ 9850 \)); n.m.r. (CDCl\(_3\)):
\[ \delta 8.60 \ (s, \ 1, \ H-8), \ 8.12 - 7.40 \ (m, \ 5, \ B_2), \ 6.80 \ (d, \ 1, \ J_{1',2'} \ 4.7 \text{ Hz}, \ H-1'), 5.46 \ (q, \ 1, \ J_{2',3'} \ 7.0 \text{ Hz}, \ H-2'), 4.89 \ (q, \ 1, \ J_{3',4} \ 6.0 \text{ Hz}, \ J_{4',5'_{a}} \ 5.0 \text{ Hz}, \ J_{4',5'_{b}} \ 4.0 \text{ Hz}, \ H-4'), 4.60 \ (d, \ 1, \ H-5'a), 4.58 \ (d, \ 1, \ H-5'b), 3.45 \ (m, \ 1, \ J_{1''_{a},3'} \ 8.0 \text{ Hz}, \ J_{1''_{b},3'} \ 3.8 \text{ Hz}, \ H-3'), 3.10 \ (q, \ 1, \ J_{1''_{a},1''_{b}} \ 18.2 \text{ Hz}, \ H-1''_{a}), 2.86 \ (q, \ 1, \ H-1''_{b}). \]


The 2,6-dichloropurine \( \beta \)-nucleoside 174 was isolated as an amorphous solid; m.p. 50-60\(^\circ\), \([\alpha]_{D}^{22} + 36.0 \ (\epsilon 1.0, \ \text{dichloromethane})\); \( \lambda_{\text{max}}^{\text{MeOH}} \ 215 \ (\epsilon 16,900), 229 \ (\epsilon 11,600), \) and 274 nm (\( \epsilon 7,110 \)); n.m.r. (CDCl\(_3\)):
\[ 8.28 \ (s, \ 1, \ H-8), \ 8.07 - 7.25 \ (m, \ 5, \ C_6H_5), \ 6.24 \ (d, \ J_{1',2'} \ 1.0 \text{ Hz}, \ H-1'), 5.64 \ (q, \ 1, \ J_{2',3} \ 6.5 \text{ Hz}, \ H-2'), 4.76 - 4.25 \ (\text{overlapping signals}, \ 3, \ H-4' \ and \ 5'), 3.71 \ (q, \ 1, \ J_{3',4} \ 8.5 \text{ Hz}, \ J_{1''_{a},3'} \ 8.0 \text{ Hz}, \ H-3'), 3.01 \ (q, \ 1, \ J_{1''_{a},1''_{b}} \ 18.0 \text{ Hz}, \ H-1''_{a}), 2.62 \ (d, \ 1, \ H-1''_{b}). \]

Molecular weight by mass spectrometry 448.0336.

C\(_{19}\)H\(_{14}\)O\(_5\)N\(_4\)Cl\(_2\) requires 448.0341.

9-[3'-C-(Carboxymethyl-2',3'-\( \gamma \)-lactone)-3'-deoxy-\( \beta \)-D-ribofuranosyl]adenine (178) and 9-[3'-C-Carboxymethyl-3'-deoxy-\( \alpha \)-D-ribofuranosyl]adenine (181).

To a solution of the lactone 169 (2.00 g) and N\(^6\)-benzoyl-N\(^6\),9-bis(trimethylsilyl)adenine (2.50, 1.04 equiv) in dichloroethane (60 ml) was added a solution of tin
tetrachloride (1.46 ml, 2.0 equiv) in dichloroethane (10 ml). The solution was stirred for 18 h at 63° and after cooling to 0°, a saturated solution of sodium bicarbonate (10 ml) was added with vigorous stirring. The solution was filtered, the precipitate washed with chloroform (2 x 20 ml) and the organic and aqueous phases were separated. The organic phase was dried over sodium sulphate prior to evaporation to yield a white solid (2.2 g). Column chromatography of the product on silica gel (30 g, 2.2 x 19 cm), using 9:1 ethyl acetate-ethanol as developer, afforded $^6$-benzoyl-9-[3'-(carboxymethyl-2',3'-γ-lactone)-3'-deoxy-β-D-ribofuranosyl]adenine (176) (966 mg, 31%) and $^6$-benzoyl-9-[3'-(carboxymethyl-2',3'-γ-lactone)-3'-deoxy-α-D-ribofuranosyl]adenine (177) 954 mg, 31%). Both nucleosides were used for subsequent reaction without further purification.

The β-nucleoside 176: n.m.r. (CDCl$_3$): δ 9.52 (s, 1, NH), 8.56 and 8.10 (2s, 2, H-2 and 8), 7.95 - 7.31 (overlapping signals, 10, C$_6$H$_5$), 6.20 (s, 1, H-1'), 5.65 (d, 1, J$_2$,3$, 6.8 Hz, H-2'), 4.70 (q, 1, J$_4$'$, 5'$a 5.8 Hz, J$_5'$a,5'$b 12.0 Hz), 4.48 (q, 1, J$_4$'$, 5'$b 5.4 Hz, H-5'b), 4.27 (qn, 1, J$_3$,4$, 8.4 Hz, H-4'), 3.80 (q, 1, J$_1$"a, 3', 9.0 Hz, H-3'), 2.96 (q, 1, J$_1$"a,1"b 18 Hz, H-1"a), 2.60 (d, 1, H-1"b).

The α-nucleoside 177: n.m.r. (CDCl$_3$): δ 9.43 (s, 1, NH), 8.70 and 8.25 (2s, 2, H-2 and 8), 8.30 - 7.40 (overlapping signals, 10, C$_6$H$_5$), 6.89 (d, 1, J$_1$',2', 4.0 Hz, H-1'), 5.32 (q, 1, J$_2$,3$, 7.0 Hz, H-2'), 4.77 (q, 1, J$_3$,4$, 6.0 Hz, J$_4$,5'a 5.0 Hz, J$_4$,5'b 4.0 Hz, H-4'), 4.57 (q, 1,
To a solution of the β-nucleoside 176 (210 mg) in anhydrous methanol (16 ml) was added a solution of sodium methoxide (0.66 ml of a 5 mg sodium/ml methanol solution; 0.33 equiv) and the mixture was maintained at room temperature for 7 days. Bio-Rex 70 (H+)) cation exchange resin (48 mg, 1.1 equiv.) was added to the solution and the mixture was stirred for 15 min. before the resin was removed by filtration. The filtrate was then evaporated under reduced pressure to afford an amorphous solid. The product was then chromatographed on a column of Bio-Rex 70 (H+) resin (1 x 11 cm), using 8:2 methanol-water as developer, to afford the lactone β-nucleoside 178 (76 mg, 61%) which crystallized spontaneously from solution. An analytical sample of compound 178 was recrystallized from water; m.p. 196-198°, [α]D +24 -29° (c 0.09, methanol); νKBr max 1780 cm⁻¹ (C=O); λMeOH max 205 (ε 18,700), 257 nm (ε 13,200); c.d. Δε -1.82 (λmax 260 nm, c 0.0003, methanol); n.m.r. (DMSO-d₆): 8.45 and 8.15 (2s, 2, H-2 and H-8), 7.31 (s, 2, exchanges in D₂O, NH₂), 5.91 (s, 1, H-1'), 5.21 (br s, 1, exchanges in D₂O, OH), 4.45 (d, 1, J₂',₃', 4.8 Hz, H-2'), 3.93 (s, 1, J₃',₄', 9.6 Hz, J₄',₅'b 3.3 Hz, J₄',₅'a 2.7 Hz, H-4'), 3.78 (q, 1, J₅'a,₅'b 13.0 Hz, H-5'a), 3.59 (q, 1, H-5'b), 2.66 (sept, 1, J₃',₇'a 9.6 Hz, J₃',₇'b 4.8 Hz, H-3'), 2.53 (q, 1, J₁''a,₁''b 1.7 Hz, H-1''a),
2.50 (m, DMSO), 2.33 (q, 1, H-1"b).

Anal. Calc. for C$_{12}$H$_{13}$N$_5$O$_4$·1 1/2 H$_2$O: C, 45.28; H, 5.07; N, 22.00. Found: C, 45.60; H, 5.07; N, 21.97. Molecular weight by mass spectrometry 291.0953. C$_{12}$H$_{13}$N$_5$O$_4$ requires 291.0967. M$^+$ + 1 292.1032 required 292.1045.

To a solution of the α-nucleoside 177 (349 mg) in anhydrous methanol (22 ml) was added a solution of sodium methoxide (0.90 ml of a 5 mg sodium/ml methanol solution; 0.37 equiv) and the mixture was maintained at room temperature for 7 days. Bio-Rex 70 (H$^+$) cation exchange resin (64 mg, 0.9 equiv) was added to the solution and the mixture was stirred for 15 min after which the resin was removed by filtration. The filtrate was evaporated in vacuo to afford an amorphous white solid which was recrystallized from methanol to afford 9-((3'-C-carbomethoxymethyl-3'-deoxy-α-D-ribofuranosyl)adenine (179); m.p. 183.0 - 183.5°, $[\alpha]_D^{22}$ = +20° (c 0.5, 1:1 methanol-water).

Anal. Calc. for C$_{13}$H$_{17}$N$_5$O$_5$: C, 48.29; H, 5.30; N, 21.66. Found: C, 48.29; H, 5.48; N, 21.36.

Compound 180 was dissolved in a minimum amount of 8:1 methanol-water and the solution was applied to a column of Bio-Rex 70 (H$^+$) resin (1 x 12 cm) which was packed and eluted with 8:2 methanol-water. The acidic α-nucleoside 181 crystallized spontaneously from the eluant solution and was removed by filtration to afford a white crystalline solid (106 mg, 51%); m.p. 233-236°, $[\alpha]_D^{24}$ 0° (c 0.1, 1:1
methanol-water); $\lambda_{\text{max}}^2$H$_2$O 203 (ε 17,900), 252 nm (ε 12,700);
$v_{\text{max}}^\text{KBr}$ 2400 - 3600 cm$^{-1}$ (OH); c.d. $\Delta \varepsilon$ +1.53 ($\lambda_{\text{max}}$ 256 nm, C 0.0003, water); n.m.r. (DMSO-d$_6$); 12.13 (br s, 1, exchanges in D$_2$O, CO$_2$H), 8.13 and 8.09 (2s, 2, H-2 and 8), 7.25 (s, 2, exchanges in D$_2$O, NH$_2$), 6.27 (d, 1, J$_1'$, 2', 3.0 Hz, H-1'), 5.57 (br s, 1, exchanges in D$_2$O, OH), 4.83 (br s, 1, exchanges in D$_2$O, OH), 4.26 (t, 1, J$_2'$, 3', 3.8 Hz, H-2'), 4.03 (sex, 1, J$_3'$, 4', 9.5 Hz, J$_4'$, 5', b 4.0 Hz, J$_4'$, 5', a 3.0 Hz, H-4'), 3.63 (q, 1, J$_5'$, a, 5', b 12.0 Hz, H-5'a), 3.47 (q, 1, H-5'b), 2.68 (oct, 1, J$_1$''b, 3 3.2 Hz, H-3''), 2.61 (d, 1, J$_1$'a, 1''b 16.0 Hz, H-1'a), 2.50 (m, DMSO), 2.39 (q, 1, H-1''b).

Anal. Calc. for C$_{12}$H$_{15}$N$_5$O$_5$: C, 46.60; H, 4.85; N, 22.65. Found: C, 47.65; H, 4.99; N, 22.66. Molecular weight by mass spectroscopy 291.0984. C$_{12}$H$_{13}$N$_5$O$_4$ (M$^+$ - H$_2$O) requires 291.0968. (M$^+$ + 1) - H$_2$O 292.1041 required 292.1046.
4. **Synthesis of Glycos-3-yl α-Amino Acids and Analogues of the Nucleoside Moiety of Polyoxin J.**

(E) and (Z)-5-O-Benzoyl-3-deoxy-1,2-O-isopropylidene-3-C-[nitro(methoxycarbonyl)methylene]-α-D-erythro-pentofuranose (189).

To a solution of 5-O-benzoyl-1,2-O-isopropylidene-α-D-erythro-pentos-3-ulose (182, 554 mg) and ammonium acetate (162 mg, 1.1 equiv.) in anhydrous N,N-dimethylformamide (3 ml) was added methyl nitroacetate (468 mg, 2.1 equiv.). After the reaction mixture was stirred at room temperature for 2 h, it was filtered, the filtrate was diluted with chloroform (25 ml) and the solution washed with water (3 x 10 ml), dried over sodium sulphate, and evaporated to yield an amber syrup. T.l.c. of the product showed the presence of two components with R_f values of 0.35 and 0.45 when 8:2 benzene-ethyl acetate was used as the developer. The crude product mixture was dissolved in acetic anhydride (10 ml) and to the resulting solution was added p-toluenesulphonic acid monohydrate (90 mg). The reaction mixture was heated to ~90° for 5 h at which time t.l.c. of the product showed the presence of only the higher R_f component. The solution was evaporated in vacuo and the resulting crude product was dissolved in chloroform (25 ml). The chloroform solution was then washed with water (3 x 10 ml), dried over sodium sulphate, and evaporated to yield a dark gummy solid (547 mg).
Column chromatography of the crude product on silica gel (30 g), using 9:1 benzene-ethyl acetate as the developer, afforded the title compound as a pale yellow syrup (216 mg, 29%). An analytical sample of compound 189 was obtained by distillation at 140-160° and 0.06 torr; \([\alpha]_D^{26} + 323°\) (c 1.6, chloroform); \(\nu_{\text{max}}^{\text{CHCl}} 1730 (\text{C=O})\) and 1545 cm\(^{-1}\) (NO\(_2\)); n.m.r.* (CDCl\(_3\)): \(\delta 8.1 - 7.2\) (overlapping signals, 5, C\(_6\)H\(_5\)), 6.05 (d, 1, J\(_{1B,2B}\) 4.5 Hz, H-1B), 6.02 (d, 1, J\(_{1A,2A}\) 4.8 Hz, H-1A), \(\sim 6.00\) (overlapping signals, 1, J\(_{2A,4A}\) 1.8 Hz, J\(_{4A,5A}\) 1.2Hz, H-4A), 5.86 (m, 1, J\(_{2B,4B}\) 1.8 Hz, J\(_{4B,5B}\) 3 Hz, H-4B), 5.67 (q, 1, H-2B), 5.52 (q, 1, H-2A), 4.60 and 4.57 (2d, 2, H-5A), 4.52 and 4.46 (2d, 2, H-5B), 3.94 (s, 3, CO\(_2\)CH\(_3\)-B), 3.90 (s, 3, CO\(_2\)CH\(_3\) - A), 1.44 and 1.40 (2s, 6, CH\(_3\)).

Anal. Calc. for C\(_{18}\)H\(_{19}\)NO\(_9\): C, 54.96; H, 4.87; N, 3.56. Found: C, 55.00; H, 5.00; N, 3.36

* The n.m.r. spectrum was of a mixture of geometrical isomers. The major to minor product ratio, calculated by the relative heights of the methyl ester resonances at \(\delta 3.90\) and 3.94, respectively, was 1.9:1. Those resonances attributed to the major isomer are labelled A and to the minor isomer B.
1,2:5,6-Di-O-isopropylidene-3-C-[(R,S)-nitro(methoxycarbonyl)methyl]-α-D-allofuranose (162).

To a mixture of 1,2:5,6-di-O-isopropylidene-α-D-ribo-hexofuranos-3-ulse (25) (1.00 g, 1.0 equiv.), ammonium acetate (0.30 g, 1.0 equiv.) and anhydrous N,N-dimethylformamide (4 ml) was added dropwise, with stirring, methyl nitroacetate (0.92 g, 2.0 equiv.). After the reaction mixture was stirred for 20 min at room temperature, the solution was filtered, water was added (4 ml) and the reaction mixture was extracted with chloroform (3 x 10 ml). The combined organic extracts were dried (magnesium sulphate), and evaporated to yield an orange syrup (1.40 g). The crude product was chromatographed on silica gel (150 g, 5 x 17 cm, 1:1 benzene-ethyl acetate as developer) to afford starting material 25 (157 mg, Rf 0.44), and a 1:5 mixture (1 g, Rf 0.58) of methyl nitroacetate and product 162:

$\nu_{\text{max}}^\text{film}$ 3450 (OH), 1755 cm$^{-1}$ (C=O); n.m.r. (CDCl$_3$): δ 5.90 (d, 1, J$_{1,2}$ 4 Hz, H-1), 5.83 (s, 1, H-1'), 5.18 (s, CH$_2$ of methyl nitroacetate), 3.88 (s, OCH$_3$ of methyl nitroacetate), 3.83 (s, 3, CO$_2$CH$_3$), 3.47 (s, 1, OH, exchanges with D$_2$O), 1.46, 1.37 and 1.33 (3s, 12, CH$_3$).

3-O-Acetyl-1,2:5,6-di-O-isopropylidene-3-C-[(R,S)-nitromethoxycarbonyl)methyl]-α-D-allofuranose (165).

Methyl nitroacetate (4.9 g, 1.9 equiv.), ketose 25 (5.7 g, 1.0 equiv.), and ammonium acetate (1.7 g, 1.0 equiv.)
were reacted in N,N-dimethylformamide (20 ml) as previously described. The crude product obtained and p-toluene-
sulphonic acid monohydrate (0.8 g) were dissolved in acetic anhydride (40 ml) and stirred for 5 h at 80-85°C. The acetic anhydride was removed by co-distillation with toluene (3 x 30 ml) under reduced pressure to yield a dark brown syrup (9.0 g). The crude product was chromatographed on silica gel (200 gm, 5 x 24 cm, 8:2 benzene-ethyl acetate as developer) to yield a clear, pale yellow syrup (Rf 0.37). Compound 165 was crystallized from ether-hexane to afford a white crystalline solid (7.5 g, 82% based on ketose 25); m.p. 136-137°, [α]D24 + 71.2° (c 5.3, chloroform); νCHCl3 max 1740 (C=O), and 1570 cm⁻¹ (NO₂); n.m.r. (CDCl₃): δ 5.93 (d, 1, J₁,₂ 3.5 Hz, H-1), 5.90 (s, 1, H-1'), 4.90 (d, 1, H-2), 4.87 (s, 1), 4.34 (s, 3), 3.80 (s, 3, CO₂CH₃), 2.08 (s, 3, OAc), 1.50, 1.43, 1.37, and 1.33 (4s, 12, CH₃).

Anal. Calc. for C₁₇H₂₅NO₁₁: C, 48.69; H, 6.01; N, 3.34. Found: C, 48.86; H, 6.08; N, 3.10.

Methyl N-acetyl-α-D-2-(1,2:5,6-di-O-isopropylidene-α-D-glucofuranos-3-yl)glycinate (202) and Methyl N-acetyl-D-
2-(1,2:5,6-di-O-isopropylidene-α-D-glucofuranos-3-yl)
glycinate (203).

To a pre-hydrogenated mixture of palladium-on-charcoal (0.95 g) in methanol (100 ml) was added a solution of the nitro ester 162 (2.3 g) dissolved in a minimum of methanol.
Acetic anhydride (6 ml) was added and the reaction mixture hydrogenated at atmospheric pressure for 18 h. The solution was filtered, neutralized with aqueous sodium bicarbonate solution, and extracted with chloroform (5 x 100 ml) to yield a pale yellow syrup (1.7 g). Column chromatography of the crude product on silica gel (300 g) with 10:5:1 benzene-ether-ethanol as developer afforded 202 (0.27 g, 12%) and 203 (0.20 g, 9%) as pale yellow syrups, as well as ketose 25 and two unidentified compounds (0.22 g and 0.24 g).

Compound 202 was rechromatographed on silica gel (32 g, 1.8 x 30 cm) with 8:2 ethyl acetate-ethanol as developer to yield a clear colourless syrup (0.17 g) which was distilled at 130° and 0.2 torr to yield a clear hard glass; m.p. 52-62°, [α]_D^{25} + 71° (c 1.5, methylene chloride); n.m.r. (CDCl₃):

6 6.92 (br d, 1, J_NH,1' 6.5 Hz, NHAc), 5.83 (d, 1, J_1,2 3.5 Hz, H-1), 4.97 (d, 1, H-1'), 4.35 (d, 1, H-2), 4.30 (br s, 1, OH, exchanges in D₂O), 3.78 (s, 3, CO₂CH₃), 2.04 (s, 3, NAc).


Compound 203 (0.20 g) was rechromatographed on silica gel (40 g, 1.8 x 38 cm) with 8:2 ethyl acetate-ethanol as developer to yield a clear, colourless syrup (0.12 g); [α]_D^{27} + 50.6° (c 1.1, methylene chloride); n.m.r. (CDCl₃):
δ 6.90 (br d, 1, J\text{NH,H-1}, 10 Hz, NHAc), 5.89 (d, 1, J\text{1,2}, 4 Hz, H-1), 5.18 (d, 1, H-1'), 4.48 (d, 1, H-2), 4.28 (s, 1, OH, exchanges in D\text{2}0), 3.80 (s, 3, CO\text{2}CH\text{3}), 2.00 (s, 3, NAc).

Molecular weight by mass spectroscopy 374.1469. C\text{16}H\text{24}NO\text{9} (M\text{+}-CH\text{3}) required 374.1449.

Chromatographic investigations of the two unidentified fractions of the initial chromatography showed that the first (0.22 g) was a mixture of at least six components while the second (0.24 g) was a mixture of three components, all of which remain unidentified.

3-O-Acetyl-1,2:5,6-di-O-isopropylidene-3-C-(methoxydi-carbonyl)-α-D-allofuranose oxime (204).

The nitro ester 165 (165 mg) in methanol (20 ml) was hydrogenated at atmospheric pressure, with palladium-on-charcoal as catalyst (5%, 150 mg), for 48 h. The catalyst was removed by filtration and the filtrate evaporated to yield the oxime 204 (148 mg, 94%) as a clear colourless syrup. An analytical sample was obtained by distillation at 110-120°/0.08 torr; [\alpha]^{23}_{D} + 10.7° (c 1.2, chloroform) n.m.r. (CDCl\text{3}): δ 5.89 (d, 1, J\text{1,2}, 3.5 Hz, H-1), 5.5 - 4.9 (br s, 1, exchanges in D\text{2}0, NOH), 4.6 - 4.1 (overlapping signals, 4, H-4, 5, and 6), 4.42 (d, 1, H-2), 3.82 (s, 3, CO\text{2}CH\text{3}), 2.09 (s, 3, OAc), 1.46 and 1.34 (2s, 12, CH\text{3}).

Anal. Calc. for C\text{17}H\text{25}NO\text{10}: C, 50.62; H, 6.25; N, 3.47. Found: C, 50.49; H, 6.58; N, 3.23.
Methyl L-2-(3-O-acetyl-1,2:5,6-di-O-isopropylidene-\(\alpha\)-D-allofuranos-3-yl)glycinate (205) and Methyl D-2-(3-O-acetyl-1,2:5,6-di-O-isopropylidene-\(\alpha\)-D-allofuranose-3-yl)glycinate (206).

The 3-O-acetyl nitro ester 165 (200 mg) in methanol (12 ml) was hydrogenated over freshly activated Raney nickel (0.4 ml) catalyst for 4 h at atmospheric pressure and room temperature. The catalyst was removed by filtration and the filtrate evaporated to yield a clear colourless syrup (150 mg, 81%). Column chromatography of the product on silica gel (30 g, 2.3 x 22 cm), with 9:1 ethyl acetate-ether as developer, yielded two pure, ninhydrin positive, compounds.

Compound 205 (\(R_f\) 0.35, 124 mg, 67%) was distilled at 100-110° and 0.05 torr; \([\alpha]_{D}^{24} + 38.4^\circ\) (c 0.9, methylene chloride); n.m.r. (CDCl\(_3\)): \(\delta\) 5.81 (d, 1, J\(_{1,2}\) 3.5 Hz, H-1), 4.70 - 4.14 (overlapping signals, 5), 4.41 (d, H-2), 4.11 (br s, 1, H-1', collapses to sharp singlet after addition of D\(_2\)O), 3.66 (s, 3, CO\(_2\)CH\(_3\)), 2.00 (s, 3, OAc), 1.83 (br s, 2, NH\(_2\), exchanges in D\(_2\)O), 1.48 (s, 9, CH\(_3\)), 1.34 (s, 3, CH\(_3\)).

Anal. Calc. for C\(_{17}\)H\(_{27}\)NO\(_9\): C, 52.44; H, 6.99; N, 3.60. Found: C, 52.73; H, 6.87; N, 3.46.

Compound 206 (\(R_f\) 0.42, 15 mg, 8%) was distilled at 120° and 0.2 torr; \([\alpha]_{D}^{23} + 16.6^\circ\) (c 1.7, methylene chloride); n.m.r. (CDCl\(_3\)): \(\delta\) 5.88 (d, 1, J\(_{1,2}\) 4 Hz, H-1), 4.68 (d, 1,
J, T. 2 Hz, H-4), 4.58 (d, 1, H-2), 4.28 (s, 3), 4.02 (br. s, 1, H-1', sharp single after addition of D, 0), 3.76 (s, 3, CO, CH, ), 2.08 (s, 5, OAc and NH, collapses to 3 proton signal upon addition of D, 0), 1.53 (s, 3, CH, ), 1.44 (s, 6, CH, ), 1.34 (s, 3, CH, ).

Found: C, 52.39; H, 7.10; N, 3.49.

L-2-(1,2:5,6-Di-O-isopropylidene-D-allofuranos-3-yl)-glycine (207) and D-2-(1,2:5,6-Di-O-isopropylidene-D-allofuranos-3-yl)glycine (208).

To a solution of the L-glycinate 205 (50 mg) in methanol (1 ml) was added a catalytic amount of sodium hydroxide (1 ml of a 2.5% solution) and the solution was kept at room temperature for 4 h. The solution was applied to a column of Rexyn RG-51 (H+) resin (15 ml) and the column was eluted with water. The ninhydrin positive fractions were combined and evaporated to yield the amino acid 207 (35 mg, 82%) as a pale, yellow glass. Compound 207 was crystallized from ethanol-ethyl acetate; m.p. 189-191°, [α]D + 89.2° (c 0.6, water); c.d. Δε + 0.97 (λmax 209, 0.5 N HCl in methanol); n.m.r. (D, 0): δ 6.08 (d, 1, J, 2 3.5 Hz, H-1), 5.07 (d, 1, J, 4, 5 3 Hz, H-4), 4.57 (d, 1, H-2), 4.37 - 3.77 (overlapping signals, 4, H-1', 5, and 6), 1.48 and 1.30 (2s, 12, CH, ).

Anal. Calc. for C, H, N: C, 49.77; H, 7.01; N, 4.15. Found: C, 49.74; H, 7.10; N, 4.04.
Identical base hydrolysis of the D-glycinate (55 mg) yielded the D-amino acid (40 mg, 85%). Recrystallization of from ethanol-ether afforded a pure white crystalline solid; m.p. 157-159°, \( [\alpha]^2_{D} + 25^\circ (c 0.7, \text{water}) \); c.d.; \( \Delta\varepsilon = 0.93 \) (\( \lambda_{\text{min}} = 209, 0.5 \text{ N HCl in methanol} \)); n.m.r. (\( \text{D}_2\text{O} \)): \( \delta 6.07 \) (d, 1, \( J_{1,2} 3.5 \text{ Hz} \), H-1), 4.37 - 3.60 (overlapping signals, 5), 1.57, 1.53, and 1.38 (3s, 12, \( \text{CH}_3 \)).

Anal. Calc. for \( \text{C}_{14}\text{H}_{23}\text{NO}_8 \cdot \frac{1}{2}\text{H}_2\text{O} \): C, 49.12; H, 7.07; N, 4.09. Found: C, 49.03; H, 7.15; N, 4.10.

Methyl N-acetyl-L-2-(3-O-acetyl-1,2:5,6-di-O-isopropylidene-\( \alpha\)-D-allofuranos-3-yl)glycinate (209) and methyl N-acetyl-D-2-(3-O-acetyl-1,2:5,6-di-O-isopropylidene-\( \alpha\)-D-allofuranos-3-yl)glycinate (210).

Acetic anhydride (3 ml) was added to a solution of the L-glycinate (510 mg) in methanol (10 ml) and the solution was stirred for 21 h at room temperature. The solution was evaporated under reduced pressure and the residual acetic anhydride was removed by co-distillation with toluene, in vacuo, to yield 209 as an amorphous solid (579 mg, 100%). An analytical sample of the N-acetyl L-glycinate 209 was obtained by distillation at 140° and 0.2 torr; m.p. 56-59°, \( [\alpha]^2_{D} + 18.1^\circ (c 3.3, \text{methylene chloride}) \); n.m.r. (\( \text{CDCl}_3 \)): \( \delta 6.68 \) (d, 1, \( J_{\text{NH,H}-1} 9.5 \text{ Hz} \), exchanges in \( \text{D}_2\text{O} \), \( \text{NHAc} \)), 5.93 (d, 1, \( J_{1,2} 4 \text{ Hz} \), H-1), 5.45 (d, 1, collapses to singlet after addition of \( \text{D}_2\text{O} \), H-1'), 4.51 (d, 1, H-2), 4.46 - 4.00
(overlapping signals, 4, H-4, 5 and 6), 3.76 (s, 3, CO₂CH₃),
2.14 and 2.10 (2s, 6, Ac), 1.50, 1.48, 1.42 and 1.37 (4s,
12, CH₃).

**Anal. Calc. for C₁₀H₂₀NO₉:** C, 52.89; H, 6.78; N, 3.25.
Found: C, 52.68; H, 6.90; N, 3.27.

Identical treatment of D-glycinate 206 (50 mg) yielded
N-acetyl D-glycinate 210 (56 mg, 100%) as a clear colourless
syrup, which was distilled at 120° and 0.2 torr; [α]₂⁵ +
30.8° (c 3.6, chloroform); n.m.r. (CDCl₃): δ 7.00 (d, 1,
J₁₂N, H-1' 10 Hz, exchanges in D₂O, NHAc), 5.91 (d, 1, J₁₂,
3.8 Hz, H-1), 5.27 (d, 1, collapses to singlet after
addition of D₂O, H-1'), 4.59 (d, 1, H-2), 4.42 - 4.12 (over-
lapping signals, 4, H-5 and H-6), 3.78 (s, 3, CO₂CH₃), 2.06
and 2.01 (2s, 6, Ac), 1.58, 1.50, 1.47 and 1.38 (4s, 12,
CH₃).

**Anal. Calc. for C₁₀H₂₀NO₉:** C, 52.89; H, 6.78; N,
3.25. Found: C, 52.65; H, 6.81; N, 3.15.

**Methyl N-acetyl-L-2-(1,2:5,6-di-0-isopropylidene-α-D-**
allofuranos-3-yl)glycinate (211).**

To a solution of the N-acetyl L-glycinate 209 (500 mg)
in anhydrous methanol (5 ml) was added a catalytic amount
of sodium methoxide (1.5 ml of a 0.5% solution) and the
solution was kept for 25 min. The solution was decationized
with Amberlite IRC-50 (H⁺) resin and evaporated to yield a
pale yellow glass (499 mg). The product was chromatographed
on silica gel (50 g, 2 x 39 cm), with 9:1 benzene-ethyl acetate as the developer, to afford the starting material \( \text{209} \) \((R_f 0.31, 16 \text{ mg})\) and the title compound \( \text{211} \) \((R_f 0.30, 396 \text{ mg}, 91\% \text{ based on starting material consumed})\). Compound \( \text{211} \) was recrystallized from benzene-hexane; m.p. 141-142°, \([\alpha]_{D}^{24} + 27.4^\circ \text{ (c 1.7, chloroform)}\); n.m.r. \((\text{CDCl}_3)\): \(\delta 6.83 \text{ (d, 1, } J_{1'-\text{NH}} 9.5 \text{ Hz, NHAc, exchanges with D}_2\text{O}), 5.86 \text{ (d, 1, } J_{1,2} 4.0 \text{ Hz, H-1}), 5.22 \text{ (d, 1, H-1'}, \text{ collapses to a singlet upon addition of D}_2\text{O}), 4.44 \text{ (d, 1, H-2}), 4.35 \text{ (d, 1, } J_{4,5} 3.0 \text{ Hz, H-4}), 4.09 \text{ (s, 1, } J_{5,6a} 6.0 \text{ Hz, } J_{5,6b} 6.5 \text{ Hz, H-5}), 3.83 \text{ (q, 1, } J_{6a,6b} 14 \text{ Hz, H-6a}), 3.72 \text{ (q, 1, H-6b)}, 3.69 \text{ (s, 3, } \text{CO}_2\text{CH}_3), 3.09 \text{ (br s, 1, OH, exchanges with D}_2\text{O)}, 2.05 \text{ (s, 3, NAc), 1.46 (s, 6, CH}_3), 1.39 \text{ and 1.32 (2s, 6, CH}_3)\).

\text{Anal. Calc. for C}_{17}\text{H}_{27}\text{NO}_9: C, 52.43; H, 6.99; N, 3.60. Found: C, 52.08; H, 6.96; N, 3.42.}

\text{Methyl N-acetyl-D-2-(1,2:5,6-di-O-isopropylidene-\(D\)-allofuranos-3-yl)glycinate (212).}

\text{To a solution of the 3-O-acetyl-N-acetyl \(D\)-glycinate 210 (417 mg) in methanol (10 ml) was added 0.5\% sodium methoxide in methanol (0.5 ml). IRC-50 (H\(+\)) resin was added to the solution after 1 h and the mixture was stirred for an additional 15 min. The resin was removed by filtration and the filtrate evaporated, under diminished pressure, to give an amber syrup. Column chromatography of the crude}
product on silica gel (15 g, 1.2 x 2.15 cm), using 9:1
benzene-ethyl acetate as developer, gave a clear colourless
syrup (242 mg, 64%). Distillation of the syrup at 160°/ 0.2 torr. yielded the title compound 212 as a chromatographi-
cally pure syrup; [α]_D^{22} + 45.9° (c 1.2, chloroform); n.m.r.
(CDC_3) : δ 7.08 (d, 1, J_{NH,1} 10 Hz, NHAc, exchange with
D_2O), 5.85 (d, 1, J_{1,2} 3.8 Hz, H-1), 5.25 (d, 1, collapses
to s upon addition of D_2O, H-1'), 4.58 (d, 1, H-2), 4.30
(d, 1, J_{4,5} 2.5 Hz, H-4), 4.09 (sex, 1, J_{5,6} 5.5 Hz, H-5),
3.72 (s, 5, CO_2CH_3, H-6), 2.54 (br s, 1, exchanges in D_2O,
OH), 1.96 (s, 3, Ac), 1.56, 1.47, 1.46 and 1.36 (4s, 12,
CH_3).

Anal. Calc. for C_{17}H_{27}NO_9: C, 52.44; H, 6.99; N, 3.60. Found: C, 51.94; H, 6.86; N, 3.87. Molecular
weight by mass spectrometry 389.170. C_{17}H_{27}NO_9 requires
389.169. (M^+ - CH_3) 374.146. C_{16}H_{24}NO_9 requires 374.145.

Methyl N-acetyl-L-2-(6-0-acetyl-1,2-0-isopropylidene-α-
D-allofuranos-3-yl)glycinate (221).  
The N-acetyl-L-glycinate 209 (480 mg) was dissolved
in 66% aqueous acetic acid (10 ml). After the reaction
mixture was kept for 24 h at room temperature, the solution
was evaporated to yield a clear syrup (431 mg, 99%) which
crystallized spontaneously upon standing. Recrystalliza-
tion of compound 221 from ethanol-ether produced fine, white
crystals; m.p. 124.5-126.0°, [α]_D^{30} + 47.7°(c 1.2, methanol);
n.m.r. (CDCl$_3$): $\delta$ 7.23 (d, 1, $J_{NH,1}$, 8.0 Hz, NHAc), 5.83 (d, 1, $J_{1,2}$ 3.5 Hz, H-1), 5.23 (d, 1, H-1'), 4.69 (br s, 1, OH, exchanges in D$_2$O), 4.36 (d, 1, H-2), 4.50 - 3.90 (m, 5, 1 proton exchanges in D$_2$O, OH, H-4, 5, and 6), 3.75 (s, 3, CO$_2$CH$_3$), 2.08 and 2.06 (2s, 6, Ac), 1.43 and 1.29 (2s, 6, CH$_3$); n.m.r. (DMSO-d$_6$): $\delta$ 8.36 (d, 1, $J_{NH,1'}$ 9.0 Hz, NHAc, exchanges in D$_2$O), 5.80 (d, 1, $J_{1,2}$ 3.5 Hz, H-1), 5.46 (s, 1, C-3-OH, exchanges in D$_2$O), 5.28 (d, 1, $J_{5,OH}$ 5.2 Hz, exchanges in D$_2$O, C-5-OH), 5.09 (d, 1, collapses to s upon addition of D$_2$O, H-1'), 4.47 (d, 1, H-2), 4.40 - 3.80 (m, 4, H-4, 5, and 6), 3.63 (s, 3, CO$_2$CH$_3$), 3.31 (DOH), 2.03 and 1.92 (2s, 6, Ac), 1.39 and 1.28 (2s, 6, CH$_3$).

Anal. Calc. for C$_{16}$H$_{25}$NO$_{10}$: C, 49.10; H, 6.44; N, 3.58. Found: C, 49.35; H, 6.36; N, 3.63.

Methyl N-acetyl-L-2-(5,6-di-0-acetyl-1,2-0,-isopropylidene-$\alpha$-D-allofuranos-3-yl)glycinate (222).

The 6-0-acetyl L-glycinate 221 (110 mg) was dissolved in pyridine (2 ml) and acetic anhydride (1 ml) and was stirred for 24 h at 22°. Work-up of the reaction mixture in the usual manner yielded the title compound 222 (117 mg, 96%) as a clear syrup. An analytical sample of compound 222 was obtained by distillation at 160-170° and 0.3 torr; $[\alpha]_D^{23}$ + 72.5° (c 0.8, chloroform); $\nu_{max}^{CHCl_3}$ 3450 (OH, NH), 1740 (C=O), and 1690 cm$^{-1}$ (amide I); n.m.r. (CDCl$_3$): $\delta$ 6.82 (d, 1, $J_{NH,1}$', 8.5 Hz, NHAc, exchanges in D$_2$O),
5.90 (d, 1, J _{1,2} 3.8 Hz, H-1), 5.49 (oct, 1, J _{4,5} 3.5 Hz, J _{5,6a} 2.5 Hz, J _{5,6b} 7.8 Hz, H-5), 5.08 (d, 1, H-1', collapsed to singlet upon addition of D$_2$O), 4.74 (q, 1, J _{6a,6b} 12.5 Hz, H-6a), 4.46 (d, 1, H-4), 4.45 (s, 1, OH, exchanges in D$_2$O), 4.44 (d, 1, H-2), 4.13 (q, 1, H-6b), 3.79 (s, 3, CO$_2$CH$_3$), 2.09 and 2.03 (2s, 9, Ac), 1.48 and 1.31 (2s, 6, CH$_3$).

**Anal. Calc. for C$_{18}$H$_{27}$NO$_{11}$:** C, 49.88; H, 6.28; N, 3.23. Found: C, 49.66; H, 6.33; N, 3.20.

**Methyl N-acetyl-\(\alpha\)-2-(3,5,6-tri-O-acetyl-\(\alpha\)-D-allofuranos-3-yl)glycinate (223).**

To a solution of the 5,6-di-O-acetyl-\(\alpha\)-glycinate 222 (38 mg) in acetic anhydride (1 ml) was added p-toluene-sulphonic acid monohydrate (3 mg). After the reaction was stirred for 6 h at 60°, t.l.c. of the product showed the reaction to be complete. The acetic anhydride was removed by repeated co-distillation with toluene (3 x 5 ml) under reduced pressure. The resulting syrup was dissolved in chloroform (5 ml) and washed with water (3 x 1 ml). The chloroform solution was dried over sodium sulphate and evaporated to yield the tetra-acetate glycinate 223 (37 mg, 93%) as a clear, pale yellow syrup. An analytical sample of compound 223 was obtained by distillation at 160-165° and 0.3 torr; [α]$^D_{20}$ + 81.1° (c 0.6, chloroform); n.m.r.
(CDCl₃): δ 6.71 (d, 1, Jₐₙₕ, 1', 10.5 Hz, NHAc, exchanges with D₂O), 6.02 (d, 1, J₁₂, 4.0 Hz, H-1'), 5.85 (d, 1, H-1', collapses to singlet upon addition of D₂O), 5.51 (s, 1, J₂, 2.5 Hz, J₃, 2.5 Hz, J₅, 8.0 Hz, H-5), 5.12 (d, 1, H-2), 4.54 (q, 1, J₆a, 6b 12.5 Hz, H-6a), 4.46 (d, 1, H-4), 4.03 (q, 1, H-6b), 3.74 (s, 3, CO₂CH₃), 2.12, 2.10, 2.04 and 2.01 (4s, 12, Ac), 1.58 and 1.38 (2s, 6, CH₃).

Anal. Calc. for C₂₀H₂₉NO₁₂: C, 50.52; H, 6.15; N, 2.95. Found: C, 50.31; H, 6.22; N, 2.80.

Identical acetylation of the triol glycinate 221 with acetic anhydride and p-toluenesulphonic acid monohydrate yielded the fully acetylated compound 223 in an 85% yield.

Methyl N-acetyl-L-2-(1,2-0-isopropylidene-α-D-allofuranos-3-yl)glycinate (226).

Method A: The di-0-isopropylidene-L-glycinate 221 (200 mg) was dissolved in 66% acetic acid (10 ml) and left at room temperature for 28 h. Evaporation of the solution gave a pale yellow glass, which was then dissolved in methanol (10 ml). This solution was decolorized using activated charcoal, filtered, and evaporated to give a clear colourless glass in quantitative yield; n.m.r. (DMSO-d₆): δ 8.52 (d, 1, Jₐₙₕ, 1', 8.5 Hz, NHAc, exchanges in D₂O), 5.85 (d, 1, J₁₂, 3.5 Hz, H-1'), 5.70 (br s, 1, OH, exchanges in D₂O), 5.13 (d, 1, H-1', collapses to s upon addition of
D$_2$O), 5.09 (br s, 1, OH, exchanges with D$_2$O), 4.92, (br s, 1, OH, exchanges in D$_2$O), 4.52 (d, 1, H-2), 4.30 (d, 1, J$_{4,5}$ 6 Hz, H-4), 4.00 - 3.10 (m, 3, H-5, 6), 3.69 (s, 3, CO$_2$CH$_3$), 2.00 (s, 3, Ac), 1.47 and 1.36 (2s, 6, CH$_3$).

The triol L-glycinate 226 was acetylated with acetic anhydride-pyridine to yield the previously prepared methyl N-acetyl-L-2-(5,6-di-0-acetyl-1,2-0-isopropylidene-a-D-allofuranos-3-yl)glycinate (222, 214 mg, 96%).

Method B: The diol L-glycinate 221 (50 mg) was de-0-acetylated in a manner similar to that employed for the de-acetylation of the 0-acetate glycinate 209 to yield the triol L-glycinate 226 as a pale yellow glass in quantitative yield. Decolorization and acetylation of the triol 226 with acetic anhydride and pyridine yielded the 5,6-di-0-acetyl L-glycinate 222 (53 mg, 95%) as a clear colourless syrup.

Attempted Synthesis of 3-C-[(S)-Acetamino(carbomethoxy)-methyl]-1,2-0-isopropylidene-a-D-ribo-pentodialdofuranose-5,N-aminal (228).

The 3,5,6-triol L-glycinate 226 (50 mg) was dissolved in an aqueous solution (2.5 ml) of sodium periodate (32 mg, 1.0 equiv.) and sodium bicarbonate (13 mg, 1.1 equiv.). After the reaction mixture was allowed to stand for 1 1/2 h at room temperature, ethanol (3 ml) was added and the
solution was cooled to 0°. The solution was filtered and the filtrate evaporated under diminished pressure to yield a clear colourless syrup (53 mg). The crude product was used directly in subsequent reactions.

Attempted Acetylation to give 5-0-Acetyl-3-C-[(S)-acetamino(carbomethoxy)methyl]-1,2-0-isopropylidene-α-D-ribo-pentodialdofuranose-5,N-aminal (229).

The above product was dissolved in acetic anhydride (1 ml) and pyridine (2 ml) and stirred for 24 h at room temperature. Work up in the usual manner gave a light brown syrup (90 mg). Column chromatography of the crude product on silica gel (10 g, 14 x 1.5 cm), with 8:2 benzene-ethanol as developer, yielded a mixture of greater than five components in low yield.

Attempted Acetylation to give 3-C-[(S)-Acetamino(carbomethoxy)methyl]-3,5-di-0-acetyl-1,2-0-isopropylidene-α-D-ribo-pentodialdofuranose-5,N-aminal (230).

The crude product obtained from the oxidation of the triol was dissolved in acetic anhydride (1 ml) and to this was added p-toluenesulphonic acid monohydrate (7 mg). The resulting solution was heated for 5 h at 83°. The reaction was worked-up in the usual manner and the resulting dark brown syrup (105 mg) was chromatographed on a column of silica gel (10 gm, 14.0 x 1.5 cm), with 8:2
benzene-ethanol as developer, to afford six inhomogeneous components.

**Attempted Benzoylation to give** 3-C-[(S)-Acetamino(carbomethoxy)methyl]-3,5-di-0-benzoyl-1,2-0-isopropylidene-α-D-ribo-pentodialdofuranose-5,N-aminal (231).

The crude product obtained from the periodate oxidation of the triol was dissolved in a mixture of anhydrous benzene (1 ml), pyridine (0.046 ml), and benzoyl chloride (0.038 ml). After the reaction mixture was allowed to stand for 24 h, the solution was passed through a short column of grade II alumina (0.5 g) and the column was subsequently rinsed with an additional 5 ml of anhydrous benzene. Evaporation of the eluant and column chromatography of the resulting pale yellow syrup (60 mg) on silica gel (10 gm, 14 x 1.5 cm), with 9:1 benzene-ethyl acetate as the developer, gave an inseparable mixture of compounds.

Methyl N-acetyl-L-2-(5-0-acetyl-1,2-0-isopropylidene-α-D-ribofuranos-3-yl)glycinate (233) and Methyl N-acetyl-L-2-(1,2-0-isopropylidene-α-D-ribofuranos-3-yl)glycinate (232).

The di-0-isopropylidene L-glycinate was hydrolyzed as previously described to afford the triol L-glycinate. Without further purification the crude triol was dissolved in water (10 ml) and methanol (5 ml) and a
solution of sodium periodate (109 mg) and water (5 ml) was slowly added over a period of 15 min. Ethylene glycol (2 drops) was added to consume the unreacted sodium periodate and after 5 min sodium borohydride (10 mg) was added to the solution. The resulting solution was stirred for 15 min at room temperature before adding acetone (1 ml). After a further 15 min the solution was evaporated, in vacuo, and to the resulting white solid was added ethanol (5 ml). The resulting mixture was cooled to 0°, filtered through sintered glass, and the filtrate evaporated to yield a colourless glass. The crude product was dissolved in acetic anhydride (1 ml) and pyridine (2 ml) and stirred for 24 h at room temperature. The reaction was worked-up in the usual manner to yield a tan-coloured solid which was chromatographed on a column of silica gel (20 mg), using 8:2 benzene-ethanol as the developer, to afford the 5-0-acetyl glycinate 233 (21 mg, 13%) and the 3,5-diol glycinate 232 (38 mg, 26%). Quantitative conversion of the diol 232 into its 5-0-acetyl derivative 233 was accomplished by continuing the acetylation reaction for an additional 48 hours.

An analytical sample of compound 233 was obtained by distillation at 150° and 0.20 torr; [a]_D^{24} + 57° (c 0.4, chloroform); n.m.r. (CDCl₃): δ 6.79 (br d, 1, J_NH, 8.5 Hz, exchanges in D₂O, NH), 5.95 (d, 1, J₁₂, 3.8 Hz, H-1), 5.07 (d, 1, H-1'), 4.47 (d, 1, H-2), 4.6 - 4.2 (overlapping signals, 4), addition of D₂O reduces integration to 3 protons, H-4, H-5, and OH), 3.80 (s, 3, CO₂CH₃), 2.12 and 2.08 (2s, 6, Ac), 1.50 and 1.32 (2s, 6, CH₃).

An analytical sample of the 3,5-diol $\text{L}$-glycinate 232 was obtained by distillation at 150° and 0.02 torr; $[\alpha]^D_24 + 44^\circ$ (c 1.0, chloroform); n.m.r. (CDCl$_3$): $\delta$ 6.95 (br d, 1, $J_{\text{NH,1'}}$, 9.0 Hz, exchanges in D$_2$O, NH), 5.95 (d, 1, $J_{1,2}$ 3.7 Hz, H-1), 5.57 (br s, 1, exchanges in D$_2$O, OH), 5.20 (d, 1, collapses to singlet upon addition of D$_2$O, H-1'), 4.60 - 3.95 (overlapping signals, 5, addition of D$_2$O reduces integration to 4 protons, H-2, 4, 5 and OH), 3.80 (s, 3, CO$_2$CH$_3$), 2.10 (s, 3, NAc), 1.52 and 1.34 (2s, 6, CH$_3$).


**Attempted Dehydration of Methyl N-acetyl-$\text{L}$-2-(5,6-di-O-acetyl-1,2-0-isopropylidene-$\alpha$-$\text{D}$-allofuranos-3-yl)glycinate (222).**

To a solution of compound 222 (386 mg) in pyridine (12 ml) (kept at -25°) was added thionyl chloride (4 ml). The solution was maintained at +5° overnight at which time the excess thionyl chloride was decomposed by the addition
of pyridine-water (5:1, 2 ml). Water (10 ml) was added and the aqueous solution was then extracted with chloroform (5 x 10 ml). The combined organic extracts were dried (Na₂SO₄) and evaporated to give a dark amber syrup. The unstable crude product was immediately hydrogenated in methanol over Raney nickel for 24 h. The catalyst was removed by filtration and the filtrate evaporated to yield a syrup which was chromatographed on a column of silica gel (15 g, 1.5 x 25 cm), using 8:2 benzene-ethanol as the developer, to yield the starting compound 222 (112 mg, 29%).

3-Deoxy-1,2:5,6-di-O-isopropylidene-3-C-[\((R,S)\)-nitro-\((\text{methoxycarbonyl})\text{methyl}\)]-\(\alpha\)-D-allofuranose (236).

The ketose 25 (4.67 g), methyl nitroacetate (4.30 g, 2.0 equiv.) and ammonium acetate (1.39 g, 1.0 equiv.) were reacted together in N,N-dimethylformamide (20 ml) as previously described to yield the methyl nitroacetate adduct 162, as an amber syrup. The crude product and p-toluenesulphonic acid monohydrate (0.40 gm) were dissolved in acetic anhydride (40 ml) and the temperature of the solution was quickly raised to 120° where it was maintained for 1 h. The reaction was worked-up in the usual manner to yield a thick black syrup. Column chromatography of the crude product on silica gel (500 g, 6 x 45 cm), developed with 8:2 benzene-ethyl acetate, gave the nitro
ester 165 (R_f 0.38, 4.78 gm, 63%) and (E) and (Z)-1,2:5,6-di-0-isopropylidene-3-C-[nitro(methoxycarbonyl)methylene]-α-D-ribo-hexofuranose (235) (R_f 0.42, 262 mg, 4%); n.m.r.* (CDCl_3): δ 5.89 (d, 1, J_{1A,2A} 5.0 Hz, H-1A), 5.87 (d, 1, J_{1B,2B} 4.8 Hz, H-1B), 5.69 (q, 1, J_{2A,4A} 2 Hz, J_{4A,5A} 4 Hz, H-4A), 5.62 (q, 1, J_{2B,4B} 2 Hz, J_{4B,5B} 5 Hz, H-4B), 5.50 (q, 1, H-2A and H-2B), 4.25 - 3.40 (overlapping signals, 3, H-5 and 6), 3.98 (s, 3, CO_2CH_3-B), 3.94 (s, 3, CO_2CH_3-A), 1.39, 1.37, 1.30 and 1.26 (4s, 12, CH_3).

Compound 235, without further purification, was dissolved in a solution of methanol (20 ml) and bromocresol green and the pH of the solution was adjusted to ~4.0 by addition of 1% hydrochloric acid in methanol. Sodium cyanoborohydride (46 mg, 1.0 equiv.) was added to the solution and the pH was maintained at ~4.0 by the periodic addition of acid. After 1 h at room temperature, acetone (1/2 ml) was added to the solution and 10 min later the solution was diluted with saturated sodium chloride solution (50 ml). The aqueous solution was extracted with chloroform (3 x 20 ml) and the combined extracts were dried (Na_2SO_4), filtered, and evaporated to yield a pale yellow syrup (244 mg, 89.5%). An analytical sample of compound 236 was prepared by distillation at 125°/0.2 torr, [α]_D^{25} + 80.1° (c 0.4, chloroform); n.m.r.* (CDCl_3): δ 5.84 (d, 1, J_{1,2} 3.8 Hz, H-1), 5.68 (d, 1, J_{1'A,3A} 6.0 Hz, H-1'A), 5.36 (d, 1, J_{1'B,3B} 9.8 Hz, H-1'B), 5.09 (t, 1,
\[ J_{2A,3A} 4.9 \text{ Hz}, \text{ H-2A}, 4.87 (t, 1, J_{2B,3B} 4.9 \text{ Hz}, \text{ H-2B}), 4.32 - 3.87 \text{ (overlapping signals, 4, H-4, 5 and 6)}, 3.82 \text{ (s, 3, CO}_2\text{CH}_3), 2.83 \text{ (oct, 1, J}_{3A,4A} 9.5 \text{ Hz, H-3A)}, 1.51, 1.48, 1.42, 1.38 \text{ and 1.30 (5s, 12, CH}_3\text{).}

**Anal. Calc. for C_{15}H_{23}NO_9:** C, 49.86; H, 6.14; N, 3.88. Found: C, 49.74; H, 6.61; N, 3.77.

* Compounds 235 and 236 were a mixture of geometrical and diastereomeric isomers, respectively. Those resonances, in the p.m.r. spectrum, attributed to the major isomer were designated A while those attributed to the minor isomer were designated B. Those resonances common to both isomers were to bear no designation. In the p.m.r. spectrum of compound 235 the ratio of isomers was calculated, on the basis of the heights of the methyl ester resonances, as A:B equals 1.5:1. In the p.m.r. spectrum of compound 236 the ratio of isomers was calculated, on the basis of the integrated areas of the H-2 protons, as A:B equals 2.2:1.

Methyl \( \text{L-2-(3-deoxy-1,2:5,6-di-0-isopropylidene-}\alpha-\text{D-}
\text{allofuranos-3-yl)glycinate (141)} \) and Methyl \( \text{D-2-(3-deoxy-1,2:5,6-di-0-isopropylidene-}\alpha-\text{D-}
\text{allofuranos-3-yl)glycinate (142)}. \)

To a solution of the nitro ester 236 (209 mg) in
methanol (25 ml) was added activated Raney nickel catalyst (1 ml) and the resulting mixture was vigorously stirred under an atmosphere of hydrogen for 48 h. The catalyst was removed by filtration and the filtrate was evaporated under reduced pressure to yield a clear colourless syrup. Chromatography of this syrup on silica gel (30 gm, 2 x 31 cm), using 9:1 benzene-ethanol as developer, gave compound 141 ($R_f$ 0.29, 88 mg, 46%) and compound 142 ($R_f$ 0.35, 74 mg, 38%) both as clear colourless syrups.

Compound 141; n.m.r. (CDCl$_3$): $\delta$ 5.78 (d, 1, $J_{1,2}$ 3.8 Hz, H-1), 4.78 (t, 1, $J_{2,3}$ 4.8 Hz, H-2), 4.5 - 3.5 (overlapping signals, 5, H-6, 5, 4, and 1'), 3.74 (s, 3, CO$_2$CH$_3$), 2.36 (qn, 1, $J_{3,4}$ 7 Hz, $J_{3,1}$, 7 Hz, H-3), 2.06 (br s, 2, NH$_2$, exchanges in D$_2$O), 1.48, 1.42, 1.38 and 1.29 (4s, 12, CH$_3$); (Lit value; n.m.r. (CDCl$_3$): $\delta$ 5.78 (d, 1, $J_{1,2}$ 4 Hz, H-1), 4.77 (t, 1, $J_{2,3}$ 4 Hz, H-2), 4.4 - 3.8 (overlapping peaks), 3.74 (s, 3, CO$_2$CH$_3$), 2.48 - 2.24 (m, H-1', clearly visible after addition of D$_2$O), 2.3 - 1.9 (NH$_2$, disappears on addition of D$_2$O), 1.38 - 1.22 (4s, 12, CH$_3$)); n.m.r. (CD$_3$OD): $\delta$ 5.77 (d, 1, $J_{1,2}$ 3.8 Hz, H-1), 4.82 (t, 1, $J_{2,3}$ 4.5 Hz, H-2), 4.32 (q, 1, $J_{3,4}$ 9.2 Hz, $J_{4,5}$ 4.5 Hz, H-4), 4.2 - 3.8 (overlapping signals, 3, H-5 and 6), 3.94 (d, 1, $J_{3,1}$', 5.8 Hz, H-1'), 3.73 (s, 3, CO$_2$CH$_3$), 2.31 (qn, 1, H-3), 1.45, 1.40, 1.32 and 1.28 (4s, 12, CH$_3$).
Compound 142; n.m.r. (CDCl$_3$): $\delta$ 5.78 (d, 1, J$_1$,2 4.0 Hz, H-1), 4.77 (t, 1, J$_2$,3 4.5 Hz, H-2), 4.5 - 3.6 (overlapping signals, 5, H-4, 5, 6 and 1'), 3.79 (s, 3, CO$_2$CH$_3$), 2.5 (br s, 3, H-3 and NH$_2$, collapses to broad signal representing 1 proton upon addition of D$_2$O), 1.54, 1.45, 1.34 and 1.30 (4s, 12, CH$_3$). (Lit. value 179; n.m.r. (CDCl$_3$): $\delta$ 5.79 (d, 1, J$_1$,2 3.8 Hz, H-1), 4.78 (t, 1, J$_2$,3 4.0 Hz, H-2), 4.5 - 6.1 (m, 5, H-1', 4, 5 and 6), 3.80 (s, 3, CO$_2$CH$_3$), 2.45 (m, 1, H-3), 1.85 (br s, 2, NH$_2$)); n.m.r. (CD$_3$OD): $\delta$ 4.24 (d, 1, J$_1$,2 3.8 Hz, H-1), 4.79 (t, 1, J$_2$,3 4.8 Hz, H-2), 6.4 - 3.6 (overlapping signals, 5, H-1', 4, 5 and 6), 3.76 (s, 3, CO$_2$CH$_3$), 2.37 (oct, 1, J$_3$,4 9.5 Hz, J$_3$,1, 6.5 Hz, H-3), 1.50, 1.41 and 1.31 (3s, 12, CH$_3$).

L-2-(3-Deoxy-1,2-0-isopropylidene-α-D-allofuranos-3-yl)-glycine (237).

The amino ester 141 (65 mg) was dissolved in methanol (1 ml) and to this was added 1 ml of sodium hydroxide solution (1.25% in methanol-water 1:1). The solution was stirred for 2 h at room temperature. The solution was then applied to a column of RG 51 (H$^+$) cation exchange resin (5 ml) and the column was eluted with water. The ninhydrin positive fractions were collected and evaporated to yield a pale yellow crystalline solid (34 mg, 62%). Recrystallization of compound 237 from methanol-water
yielded a white crystalline solid; m.p. 212-214°, $[\alpha]_D^{22} + 57.5°$ (c 0.3 water). [Lit. value 177; m.p. 213-215°, $[\alpha]_D^{22} + 60°$ (c 0.5, water)].

Methyl N-benzoyl-D-2-(3-deoxy-1,2:5,6-di-O-isopropylidene-\(\alpha\)-D-allofuranos-3-yl)glycinate (238).

To compound 142 (32 mg), dissolved in methanol (3 ml), was added benzoic anhydride (32 mg), and the resulting solution was stirred overnight at room temperature. The solution was then passed through a short column of alumina (grade II, 2 g) which was packed and eluted with anhydrous benzene. The charring fractions were collected and evaporated to yield a pale yellow glass. The crude product was chromatographed on a column of silica gel (5 g, 1 x 15.5 cm), using 6:4 benzene-ethyl acetate as the developer, to give the title compound 238 as a clear colourless glass. Compound 238 was crystallized from hexane-ethanol to give fine white needles; m.p. 145-146.5°, $[\alpha]_D^{22} + 28°$ (c 1.8, chloroform). [Lit. values 184; m.p. 138-140°, $[\alpha]_D^{22} + 26°$ (c 0.5, chloroform)]. The p.m.r. spectrum of compound 238 was superimposable with that of an authentic sample of methyl N-benzoyl-D-2-(3-deoxy-1,2:5,6-di-O-isopropylidene-\(\alpha\)-D-allofuranos-3-yl)glycinate.
3, 5 or 6-0-Acetyl-1,2-0-isopropylidene-3-C-[(R,S)-nitro-
(methoxycarbonyl)methyl]-\(\alpha\)-D-allofuranose (239).

The nitro ester 165 (1 gm) was dissolved in 66%
acetic acid (50 ml) and the solution was maintained for
5 h at 50°. The solution was then evaporated under reduced
pressure and the remaining traces of acetic acid were
removed by co-distillation with toluene, in vacuo, to
yield a pink syrup. Treatment of the syrup with activated
charcoal and refluxing methanol yielded, after evaporation,
a clear colourless syrup which crystallized spontaneously
upon standing (877 mg, 97%). Recrystallization of the
title compound 239 from benzene-hexane gave white crystal-
line needles; m.p. 115-120°, [\(\alpha\)]\textsubscript{D}\textsuperscript{24} + 43.5° (c 1.5, chloro-
form); n.m.r. (DMSO-d\textsubscript{6}): 6 6.44 (s, 1, exchanges in D\textsubscript{2}O,
OH), 6.22 (s, 1, H-1'), 5.84 (d, 1, J\textsubscript{1,2} 4.0 Hz, H-1),
5.70 (s, 1/2, exchanges in D\textsubscript{2}O, OH), 5.51 (br s, 1/2
exchanges in D\textsubscript{2}O, OH), 4.79 (d, 1, H-2), 4.60 - 3.86
(overlapping signals, 4, H-4, 5, and 6), 3.79 and 3.74
(2s, 3, CO\textsubscript{2}CH\textsubscript{3}), 2.03 (s, 3, OAc), 1.36 and 1.26 (2s,
6, CH\textsubscript{3}).

Anal. Calc. for C\textsubscript{14}H\textsubscript{21}NO\textsubscript{11}: C, 44.33; H, 5.58; N,
3.69. Found: C, 44.25; H, 5.57; N, 3.87.

(E) and (Z)-5,6-Di-0-acetyl-3-deoxy-1,2-0-isopropylidene-
3-C-[nitro(methoxycarbonyl)methylene]-\(\alpha\)-D-ribo-hexo-
furanoose (240).

Compound 239 (800 mg) was dissolved in acetic anhydride
(10 ml) and to this was added p-toluenesulphonic acid
monohydrate (100 mg). The temperature of the solution was elevated to 85° where it was maintained for 24 h. The volatile components of the solution were removed by evaporation and repeated co-distillation with toluene, in vacuo, and the resulting syrup was then dissolved in chloroform (30 ml). The chloroform solution was washed with saturated sodium bicarbonate solution (7 ml), washed with water (2 x 5 ml), dried over sodium sulphate, and evaporated to give a yellow syrup. Column chromatography of the crude product on silica gel (40 gm, 2 x 38 cm), using 8:2 benzene-ethyl acetate as the developer, yielded the title compound 240 as a clear colourless syrup (749 mg, 88%). An analytical sample of 240 was prepared by distillation at 140°/0.2 torr; [α]_D^{22} + 263° (c 0.7, chloroform); n.m.r. (CDCl₃): δ 5.96 - 5.45 (overlapping signals, 3, H-1, 2, 4), 5.15 - 4.74 (overlapping signals, 1, H-5), 4.47 - 3.99 (overlapping signals, 2, H-6), 3.88 (s, 3, CO₂CH₃), 2.06, 2.04 and 2.00 (3s, 6, OAc), 1.39 and 1.36 (2s, 6, CH₃).

Anal. Calc. for C_{16}H_{21}NO_{11}: C, 47.65; H, 5.25; N, 3.47. Found: C, 47.43; H, 5.39; N, 3.32.

5,6-Di-O-acetyl-3-deoxy-1,2-O-isopropylidene-3-C-[(R,S)-nitro(methoxycarbonyl)methyl]-α-D-allofuranose (241).

Method A: Compound 240 (257 mg) in methanol (2 ml) was
added to a solution of sodium cyanoborohydride (40 mg) and bromocresol green in methanol (3 ml). The solution was maintained at pH 4 by the addition of 0.1% hydrochloric acid in methanol for 20 min after which the solution was diluted with water (10 ml) and extracted with chloroform (4 x 10 ml). The combined organic extracts were dried over sodium sulphate, filtered, and evaporated to yield a pale yellow syrup (251 mg, 97%). An analytical sample of compound 241 was obtained by distillation at 125-140° and 0.2 torr; [α]_D^{22} + 41.9° (c 1.1, chloroform); n.m.r.* (CDCl₃): δ 5.87 (d, 1, J₁₂, 4.0 Hz, H-1'B), 5.77 (d, 1, J₁₂, 3.5 Hz, H-1'A), 5.41 (d, 1, J₁₀, 10.0 Hz, H-1'B), 5.36 (d, 1, J₁₀, 3.90 Hz, H-1'A), 4.97 (q, 1, J₂₃, 5.0 Hz, H-2A), 4.79 (q, 1, J₂ₓ, 5.5 Hz, H-2'B), 4.95 - 4.65 (overlapping signals, 1, H-4), 4.5 - 3.8 (overlapping signals, 3, H-5 and 6), 3.87 (s, 3, CO₂CH₃-B), 3.82 (s, 3, CO₂CH₃-A), 3.01 (sex, 1, J₃₄, 9.0 Hz, H-3'A), 3.1 (m, 1, H-3'B), 2.06 and 2.02 (2s, 6, OAc), 1.49 and 1.29 (2s, 6, CH₃).

Anal. Calc. for C₁₆H₂₃NO₁₁: C, 47.41; H, 5.72; N, 3.46. Found: C, 47.50; H, 5.60; N, 3.19.

Method B: Compound 236 (50 mg) was dissolved in 66% acetic acid and stirred for 6 1/2 h at room temperature. The solvent was removed by evaporation in vacuo and the resulting crude product, together with p-toluenesulphonic acid monohydrate (8 mg), was dissolved in acetic anhydride.
The solution was stirred for 24 h at room temperature and then worked-up in the usual manner to yield an amber syrup (42 mg, 75% based on compound 236). The crude product was distilled at 140°/0.2 torr to afford a compound identical in all respects to compound 241.

* The n.m.r. spectrum revealed a mixture of two isomers. The major isomer was designated "A" and the minor isomer was designated "B". A ratio of A:B = 1.35:1 was calculated from the relative intensities of the methyl ester resonances.

1,2,5,6-Tetra-0-acetyl-3-deoxy-3-C-[((R,S)-nitro(methoxycarbonyl)methyl]-α,β-D-allofuranose (243).

The nitro ester 241 (656 mg, 1.62 mmoles) was dissolved in acetic anhydride (.153 ml, 16.2 mmoles) and acetic acid (10 ml) and to this solution was added p-toluenesulphonic acid monohydrate (62 mg, 0.32 mmoles). After stirring for 2 h at 80-85°, the solution was cooled to room temperature and poured into ice-water (100 ml). The water was extracted with ether (4 x 50 ml) and the combined organic extracts were dried (sodium sulphate) and evaporated, in vacuo, to yield a pale yellow syrup (719 mg, 98%). An analytical sample of compound 243 was prepared by distillation at 150°/0.01 torr; [α]_D^{22} = 15.0° (c 0.6, chloroform);
n.m.r. (CDCl$_3$): $\delta$ 6.44 (d, 0.24, $J_{1\alpha,2\alpha}$ 4.0 Hz, H-1$\alpha$), 6.19 (d, 0.76, $J_{1\beta,2\beta}$ 2.8 Hz, H-1$\beta$), 4.7 - 3.3 (overlapping signals, 7, H-2, 3, 4, 5, 6, 1'), 3.92 and 3.84 (2s, 3, CO$_2$CH$_3$), 2.22, 2.14, 2.10, 2.08 and 2.06 (5s, 12, OAc).

Anal. Calc. for C$_{17}$H$_{23}$NO$_3$: C, 45.44; H, 5.16; N, 3.12. Found: C, 45.58; H, 5.24; N, 2.97. Molecular weight by mass spectroscopy 390.1048. C$_{15}$H$_{20}$NO$_{11}$ (M$^+$-OAc) requires 390.1036.

Attempted Nucleoside Synthesis with 1,2,5,6-Tetra-O-acetyl-3-deoxy-3-C-[(R,S)-nitro(methoxycarbonyl)methyl]-\(\alpha,\beta\)-D-allofuranose (243) and Bis(trimethylsilyl)thymine (244).

To a solution of compound 243 (183 mg) and compound 244 (220 mg, 2.0 equiv.) in anhydrous methylene chloride (6 ml) was added a solution of stannous chloride (153 mg, 68 $\mu$l, 1.4 equiv.) in methylene chloride (1 ml) and the reaction was stirred for 24 h at room temperature. The solution was then diluted with a saturated solution of sodium bicarbonate (217 mg) and filtered. The filtrate was extracted with ethyl acetate (4 x 10 ml), dried over sodium sulphate, filtered and evaporated to yield an amorphous solid. The product was then triturated with chloroform (10 ml), the mixture filtered, and the filtrate evaporated to yield the starting material 243 (105 mg). The filtered solid was shown by p.m.r. spectroscopy to be predominately thymine containing minor amounts of 243.
The above reaction was repeated using 1,2-dichloroethane as the solvent and after 5 or 24 h at 63° the reaction was worked-up with similar results. No evidence of nucleoside material could be found.

Methyl L-2-(3-O-acetyl-1,2:5,6-di-O-isopropylidene-α-D-allofuranos-3-yl)-N-trifluoroacetyl glycinate (245).

To a solution of compound 205 (7.94 g) in dichloromethane (120 ml) and pyridine (10 ml) at -10° was added trifluoroacetic anhydride (10 ml). After stirring at -10° for 0.5 h, water (50 ml) was added and vigorous stirring maintained until the solution spontaneously attained room temperature. The aqueous layer was separated and extracted with chloroform (2 x 40 ml) and the combined extracts were dried over sodium sulphate and evaporated, in vacuo, to afford a pale amber syrup. Column chromatography of the crude product on silica gel (450 g, 6 x 40 cm), using 6:4 benzene-ethyl acetate as the developer, afforded the title compound 245 as a clear colourless syrup (8.41 g, 85%). An analytical sample of 245 was obtained by distillation at 120-140° and 0.005 torr; [a]$_D^{22}$ + 5.7° ($\in$ 4.6, chloroform); n.m.r. (CDCl$_3$): δ 8.24 (br d, 1, J$_{1',NH}$ 9 Hz, NH), 6.16 (d, 1, J$_{1,2}$ 3.8 Hz, H-1), 5.88 (d, 1, H-1'), 4.75 (d, 1, H-2), 5.0 - 4.5 (overlapping signals, 4, H-4, 5 and 6), 3.70 (s, 3, CO$_2$CH$_3$), 2.07 (s, 3, OAc), 1.77, 1.73, 1.66 and 1.51 (4s, 12, CH$_3$).
Anal. Calc. for C₁₉H₂₆NO₁₀F₃:  C, 47.01; H, 5.40; N, 2.89. Found:  C, 47.21; H, 5.43; N, 3.10.

Methyl L-2-(3,5,6-tri-O-acetyl-1,2-O-isopropylidene-α-D-allofuranos-3-yl)-N-trifluoroacetyl glycinate (246).

Compound 245 (716 mg) was dissolved in 66% acetic acid (20 ml) and stirred for 48 h at room temperature. The reaction mixture was worked-up in the usual manner to yield a pale yellow syrup. Without further purification, the crude product was dissolved in a solution of acetic anhydride (20 ml) and p-toluenesulphonic acid monohydrate (100 mg) and stirred for 3 h at 85° and for an additional 5 1/2 h at 110°. The reaction was worked-up in the usual manner to give an amber syrup which was chromatographed on a column of silica gel (40 g, 2 x 32 cm), using 1:1 benzene-ethyl acetate as developer, to afford the title compound as a clear syrup (616 mg, 79%). An analytical sample of compound 246 was obtained by distillation at 130° and 0.01 torr; [α]²²̉D + 77.3° (C 2.0, chloroform); n.m.r. (CDCl₃): δ 7.64 (br d, 1, J₁',NH 10.0 Hz, exchanges in D₂O, NH), 6.05 (d, 1, J₁₂ 3.8 Hz, H-1), 5.83 (d, 1, collapses to singlet upon addition of D₂O, H-1'), 5.40 (oct, 1, J₄₅ 7.5 Hz, J₅₆b 5.5 Hz, J₅₆a 2.5 Hz, H-5), 5.12 (d, 1, H-2), 4.61 (d, 1, H-4), 4.58 (q, 1, J₆a₆b 12.5 Hz, H-6a), 4.08 (q, 1, H-6b), 3.78 (s, 3,
$\text{CO}_2\text{CH}_3$, 2.10, 2.06 and 2.03 (3s, 9, OAc), 1.56, 1.38 and 1.27 (3s, 6, CH$_3$).

**Anal. Calc. for C$_{20}$H$_{26}$NO$_{12}$F$_3$:** C, 45.37; H, 4.95; N, 2.65. Found: C, 45.17; H, 4.82; N, 2.50. Molecular weight by mass spectrometry 529.1387. C$_{20}$H$_{26}$NO$_{12}$F$_3$ requires 529.1406.

Methyl L-2-(5,6-di-O-acetyl-1,2-O-isopropylidene-\(\alpha\)-D-allofuranos-3-yl)-N-trifluoroacetyl glycinate (247).

Compound 245 (157 mg) was dissolved in 66% acetic acid (12 ml) and after 5 h at 22° the temperature was raised to 40° for an additional 4 h. The reaction was worked-up in the usual manner.

The crude product was dissolved in acetic anhydride (1 ml) and pyridine (4 ml) and left at room temperature for 13 h. The acetic anhydride and pyridine were evaporated *in vacuo* to afford a pale yellow syrup which was immediately chromatographed on a column of silica gel (15 g, 1.5 x 22 cm), using 6:4 benzene-ethyl acetate as developer. The chromatography yielded the starting material (2 mg), the tri-O-acetate 246 (49 mg, 29%), and the di-O-acetate 247 (101 mg, 65%). Compound 247 was recrystallized from ether-hexane; m.p. 141-142°, [\(\alpha\)]$_D^{22}$ + 54.4° (c 1.1, chloroform); n.m.r. (CDCl$_3$): $\delta$ 7.54 (br d, 1,
J', NH 9.0 Hz, exchanges in D₂O, NH), 5.94 (d, 1, J₁,₂ 3.8 Hz, H-1), 5.33 (sept, 1, J₅,₆b 7.0 Hz, J₄,₅ 4.5 Hz, J₅,₆a 2.2 Hz, H-5), 5.09 (d, 1, collapses to s upon addition of D₂O, H-1'), 4.69 (q, 1, J₆a,₆b 12.5 Hz, H-6a), 4.51 (d, 2, H-2 and 4), 4.20 (q, 1, H-6b), 3.93 (s, 1, exchanges in D₂O, OH), 3.83 (s, 3, CO₂CH₃), 2.13 and 2.06 (2s, 6, OAc), 1.48 and 1.32 (2s, 6, CH₃).

Anal. Calc. for C₁₈H₂₄NO₁₁F₃: C, 44.36; H, 4.96; N, 2.87. Found: C, 44.57; H, 4.86; N, 2.70.

Methyl L-2-(1,2,3,5,6-penta-O-acetyl-a,β-D-allofuranos-3-yl)-N-trifluoroacetyl glycinate (250).

To a solution of compound 246 (3.9 g) in acetic acid (78 ml) and acetic anhydride (78 ml) was added p-toluene-sulphonic acid monohydrate (1.9 g), and the resulting solution was stirred for 2 h at 110°. After the addition of chloroform (300 ml), the solution was washed with water (2 x 100 ml) and saturated sodium bicarbonate solution (2 x 125 ml), dried over sodium sulphate and evaporated in vacuo to yield a dark amber syrup. The crude product was chromatographed on silica gel (400 g), using 6:4 benzene-ethyl acetate as the developer, to yield compound 250 (2.0 g, 47%) as a bright yellow foam. An analytical sample of compound 250 was obtained by distillation at 150° and 0.01 torr; [α]D22 + 59.7° (c 1.9, chloroform); n.m.r. (CDCl₃): δ 7.56 (br d, 1, J' NH, l', 9.6 Hz, exchanges
in D$_2$O, pH), 6.42 (d, 1/3, J$_{1,2}$ 5.1 Hz, H-1a), 6.04 (s, 2/3, H-1b), 6.01 (s, 2/3, H-2b), 5.64 (d, 1/3, H-2a), 5.56 (1, H-1'), 5.34 (oct, 2/3, J$_{4,5}$ 7.2 Hz, J$_{5,6a}$ 2.6 Hz, J$_{5,6b}$ 6.1 Hz, H-5b), 5.28 (oct, 1/3, J$_{5,6a}$ 2.4 Hz, J$_{4,5}$ 5.6 Hz, J$_{5,6b}$ 6.4 Hz, H-5a), 4.68 (d, 2/3, H-4b), 4.63 (q, 2/3, J$_{6a,6b}$ 12.1 Hz, H-6ab), 4.61 (q, 1/3, J$_{6a,6b}$ 12.5 Hz, H-6aa), 4.51 (d, 1/3, H-4a), 4.02 (q, 1/3, H-6ba), 3.96 (q, 2/3, H-6bb), 3.78 and 3.76 (2s, 3, CO$_2$CH$_3$), 2.14, 2.12, 2.10, 2.08, 2.07, 2.06, 2.03 and 2.01 (8s, 15, OAc).

Anal. Calc. for C$_{21}$H$_{26}$NO$_{14}$F$_3$: C, 43.99; H, 4.57; N, 2.44. Found: C, 43.74; H, 4.31; N, 2.33.

Application of the Stannous Chloride Catalysed Silyl Hilbert-Johnson Procedure to the Condensation of Methyl L-2-(1,2,3,5,6-penta-O-acetyl-α,β-D-allofuranos-3-yl)-N-trifluoroacetyl glycinate (250) with N$^6$-Benzoyl-N$^6$,9-bis (trimethylsilyl) adenine (114) or Bis (trimethylsilyl)-thymine (244).

Attempt 1: To a solution of compound 250 (50 mg) and silylated N-benzoyl adenine 114 (40 mg, 1.2 equiv.) in anhydrous 1,2-dichloroethane (5 ml) was added a solution of stannous chloride (SnCl$_4$, 14 μl, 1.4 equiv.) in 1,2-dichloroethane (1 ml) and the mixture was heated to 70°. A flocculent precipitate was observed after 20-30 min.
After stirring for 13 h the reaction was cooled to 0° and sodium bicarbonate (13 mg) in ethanol (3 ml) was added followed by water (10 ml). The reaction was filtered, the filtrate was extracted with ethyl acetate (4 x 5 ml) and the combined extracts were dried over sodium sulphate and evaporated to yield an amber syrup. Chloroform (10 ml) was added to the crude product and the solution was refluxed for 5 min after which it was cooled, filtered and evaporated to yield the starting sugar (43 mg, 86%) as an amber syrup.

Attempt 2: The reaction was carried out as described above except that bis(trimethylsilyl)thymine (244, 1.2 equiv.) was used as the base. The starting material 250 was recovered in 80% yield.

Attempt 3: Compound 250 (200 mg), bis(trimethylsilyl)-thymine (118 mg) and stannous chloride (70 µl) were reacted together in acetonitrile (5 ml) for 12 1/2 h at 60°. The reaction was worked-up as described above to yield only starting material (190 mg, 95%).
Reaction of Methyl \( \text{L-2-(1,2,3,5,6-penta-0-acetyl-a,\(D\)-allofuranos-3-yl)-N-trifluoroacetyl glycinate (250) with Various Purine Bases by the Fusion Method.}

**Attempt 1**: A carefully dried mixture of compound 250 (52 mg), \(\text{N-benzoyl adenine (40 mg, 2 equiv.)}, and p-toluene-sulphonic acid (2 mg) were fused for 10 min at 180\(^\circ\)/6 torr after which the temperature was lowered to 160\(^\circ\) for a further 5 min. After cooling the crude product was extracted with boiling ethanol (4 ml) and the resulting solution was filtered and evaporated to yield a brown syrup. Column chromatography of the crude product on silica gel (5 g, 1.2 x 10.5 cm), using 9:1 benzene-ethanol as the developer, yielded only the starting material 250 (43 mg, 83%).

**Attempt 2**: Compound 250 (35 mg) and 2,6-dichloropurine (23 mg) were fused for 20 min at 160\(^\circ\)/6 torr and for an additional 10 min at 160\(^\circ\)/0.1 torr. The reaction was worked-up as described above to yield the starting material 250 quantitatively.

**Attempt 3**: Compound 250 (40 mg), 2,6-dichloropurine (27 mg) and p-toluenesulphonic acid (1 mg) were fused as described in Attempt 2 with no appreciable change in the results.
Attempt 4: Compound 250 (50 mg) and silylated N-benzoyl adenine 114 (67 mg) were fused for 10 min at 160°/13 torr and for an additional 10 min at 160°/6 torr. T.l.c. of the crude product obtained after work-up of the reaction showed the presence of predominantly starting material with no evidence of any U.V. active/charring components.

l,1'-Anhydro-2,3,5,6-tetra-O-acetyl-3-C-(R)-methoxy-carbonyl-1(R),l'(S)-N-trifluoroacetoepimo-β-D-allofuranose (252).

Compound 250 (52 mg) was dissolved in dichloromethane (6 ml), the solution was cooled to 0° and a slow stream of hydrogen bromide was passed through the solution for 2 1/2 h. The solvent and the residual acidic components were removed by repeated co-distillation with toluene (3 x 3 ml) at <35° and 15 torr. To the resulting syrup was added a solution of N^6-benzoyl-N^6,9-bis(trimethylsilyl)adenine (114, 62 mg) in dichloromethane (3 ml) and after removal of the solvent the resulting syrup was fused for 20 min at 160° and 15 torr. The reaction mixture was cooled to give a dark glass to which was added ethanol (10 ml) saturated with sodium bicarbonate. The solution was filtered and the solvent evaporated, in vacuo, to yield 65 mg of a dark brown amorphous solid. The crude product was chromatographed on a column of silica gel (17 g,
1.5 x 25 cm), using 6:4 benzene-ethyl acetate as the developer, to yield the starting material 250 (19 mg) and the title compound 252 (15 mg, 52% based on starting material consumed). An analytical sample of compound 252 was obtained by distillation at ~150° and 0.02 torr; 

\[ \alpha \]D + 117° ([α] 1.8, chloroform); n.m.r. (CDCl₃): δ 5.59 (2d, 1, J₁,₂ 1.5 Hz, H-1), 5.39 (2d, 1, H-2), 5.30 (sex, 1, J₄,₅ 6.5 Hz, J₅,₆a 3.9 Hz, J₅,₆b 6.2 Hz, H-5), 5.01 (d, 1, H-4), 4.76 (s, 1, H-1'), 4.44 (q, 1, J₆a,₆b 12.0 Hz, H-6a), 4.01 (q, 1, H-6b), 3.76 (s, 3, CO₂CH₃), 2.10, 2.06, 2.01 and 2.00 (4s, 12, OAc).

Anal. Calc. for C₁₉H₂₂NO₁₂F₃: C, 44.45; H, 4.32; N, 2.73. Found: C, 44.40; H, 4.25; N, 2.79. Mass spectrometry shows (M+1) peak at 514.1194. C₁₉H₂₃NO₁₂F₃ requires 514.1173. (M⁺-OCH₃) 482.0897 requires 482.0910.

l-[2',3',5',6'-Tetra-0-acetyl-3'-C-(-S)-N-trifluoroacetyl-carbomethoxy(amo)no)ethyl]-β-D-allofuranosyl|thymine (257).

An α,β-mixture of compound 250 (500 mg) was dissolved in anhydrous dichloromethane (30 ml) and the solution was cooled to 0°. A slow stream of anhydrous hydrogen bromide was passed through the cooled solution for 2 h. The solution was then evaporated and any remaining acetic acid removed by successive azeotroping with toluene (2 x 5 ml) under diminished pressure. The resulting syrup was
dissolved in anhydrous dichloromethane (2.6 ml) and added to a cooled (-70°) mixture of silver triflate (112 mg, 0.5 equiv.) in anhydrous dichloromethane (0.7 ml) and to the resulting mixture was added a solution of bis(trimethylsilyl)thymine (259 mg, 1.0 equiv.) in anhydrous dichloromethane (1.4 ml). The reaction was maintained at -70° for 2 h and then allowed to spontaneously attain room temperature. After 18 h the solution was cooled to 0° and a saturated solution of sodium bicarbonate (2 ml) added with vigorous stirring. The mixture was filtered and the precipitate washed with water (5 ml) and dichloromethane (10 ml). The dichloromethane phase was then separated, dried over sodium sulphate, and evaporated, in vacuo, to afford a yellow syrup. Column chromatography of the crude product on silica gel (60 g, 2.8 x 26 cm), using 10:5:1 benzene-ether-ethanol as developer, yielded the starting material 250 (267 mg, 54%) and the title compound (242 mg, 93% based on starting material consumed) as a glass; m.p. 91-96°, [α]_D^{22} + 51.8° (c 1.5, dichloromethane); c.d. Δε + 1.08 (λ_max 268, c 0.0005, methanol); n.m.r. (CDCl₃): δ 9.55 (s, 1, NH), 7.63 (d, 1, J_NH,1'' 10.0 Hz, NHCOCH₃), 7.24 (s, 1, H-6), 6.05 (d, 1, J_1',2' 7.2 Hz, H-1'), 5.78 (d, 1, H-2'), 5.61 (d, 1, H-1''), 5.42 (sept, 1, J_4',5' 7.5 Hz, J_5',6'a 2.2 Hz, J_5',6'b 6.0 Hz, H-5'), 4.88 (d, 1, H-4'), 4.60 (q, 1, J_6'a,6'b 12.2 Hz, H-6'a), 4.03 (q, 1, H-6'b), 3.84 (s, 3, CO₂CH₃), 2.10, 2.07, and 2.04 (3s, 12, OAc), 1.93 (s, 3, CH₃).
Anal. Calc. for C_{24}H_{28}N_{3}O_{14}F_{3}: C, 45.08; H, 4.41; N, 6.57. Found: C, 45.77; H, 4.57; N, 6.56. Molecular weight by mass spectrometry 639.1530. C_{24}H_{28}N_{3}O_{14}F_{3} requires 639.1515. M^{+}-thymyl 514.1140. C_{19}H_{23}NO_{12}F_{3} requires 514.1173.


Method A: Attempted Deacetylation with Sodium Methoxide in Methanol.

To a solution of compound 257 (44 mg, 0.07 mmoles) in anhydrous methanol (5 ml) was added a methanolic solution of sodium methoxide (0.1 ml, 0.1 equiv). After 2 hours at room temperature, t.l.c. (10:5:1 benzene-ether-ethanol) showed the majority of the starting material was still present. After 24 h, t.l.c. (water saturated n-butanol or ethanol) showed that all of the starting material had been consumed and only baseline material was evident. The reaction mixture was deionized with IRC-50 (H\(^{+}\)) cation exchange resin and evaporation of the solution, after removal of the resin by filtration, yielded an amorphous tancoloured solid (30 mg). Paper chromatography of the product (~10 mg), using 10:4:3 ethylacetate-pyridine-water as developer, revealed the presence of six components. The
components with $R_f$ values of 0.11, 0.15, 0.34, 0.65, and 0.73 were visible under U.V. light and the $R_f$ 0.11, 0.15, 0.34, and 0.81 components reacted positively with ninhydrin. The predominant $R_f$ 0.34 zone was eluted with water (25 ml) and the aqueous solution was freeze-dried to yield an amorphous solid (~3 mg). The p.m.r. spectrum of this product ($D_2O$, 270 MHz F.T.) showed it to be a complex mixture of compounds.

Similar reactions employing 2 or 6 equivalents of sodium methoxide afforded similar results.

A similar reaction employing 0.1 equivalents of sodium methoxide for 7 days afforded similar results.

Column chromatography of the crude product (~30 mg) on a column of Bio-Rex 70 ($H^+$) resin (1 x 11 cm), using water or 8:2 methanol-water as developer, afforded two components which were shown by paper chromatography to be a mixture of the $R_f$ 0.11, 0.15, and 0.34 components and the higher $R_f$ components.

**Method B: Attempted Unblocking with Methanolic Ammonia.**

The nucleoside 257 (30 mg) was dissolved in methanol (10 ml) and the solution was nearly saturated at 0° with ammonia. After keeping at room temperature for 5 days, it was evaporated to dryness to afford an amorphous solid. Although the paper chromatogram of the product (10:4:3 ethylacetate-pyridine-water) revealed only one component
(R_f 0.5) under U.V. light, the p.m.r. spectrum revealed a mixture of components.

No appreciable change was observed after prolonged (14 days) ammonolysis.

**Method C**: Attempted Unblocking with Methanolic Hydrogen Chloride.

The nucleoside (41 mg) was dissolved in methanol (10 ml) and the solution was nearly saturated at 0° with hydrogen chloride. After the solution had stood for 18 hours at room temperature, the solvent was evaporated, in vacuo, to yield an amber coloured glass. The above product was dissolved in water (1/2 ml) and the solution was treated with sodium bicarbonate (6 mg) in water (1 ml). A portion of the product was chromatographed on paper (10:4:3 ethylacetate-pyridene-water) and the single component detected under U.V. light was eluted with water. The p.m.r. spectrum of the product (D_2O, 270 MHz F.T.) revealed a mixture of partially acetylated compounds.

**Method D**: Attempted Deacetylation with Triethylamine.

A solution of 257 (50 mg) in methanol (5 ml) was treated with triethylamine (0.5 ml) for 24 h at room temperature. After the solvent was evaporated, in vacuo, the residue was dissolved in water and treated with IRC-50 (H^+) resin to remove the residual triethylamine. The
resin was removed by filtration and the solution evaporated to afford a glass which was shown (p.m.r. spectroscopy) to be a mixture of partially acetylated compounds.

No improvement was noted when methanol-triethylamine-water 2:1:1 was substituted as the solvent.
REFERENCES

7. (a) R.B. Duff, Biochem. J., 94 (1965) 768; 


(b) J.D. Dutcher, ibid., 18 (1963) 259.


99. (a) S.W. Gunner, W.G. Overend and N.R. Williams, Chem. Ind. (London), (1964) 1523;


112. (a) B. Helferich and R. Gootz, Ber., 62 (1929) 2788;
     (b) B. Helferich and E. Schmidz-Hillebrecht, Ber., 66 (1933) 378;
     (c) B. Helferich and U. Lampert, Ber., 67 (1934) 1167;
     (d) B. Helferich and L. Forstoff, Ber., 94 (1961) 158.


(b) L. Pichat, P. Dufay and Y. Lamorre, Compt. rend., 259 (1964) 2453;


(c) A. Endo, K. Kakiki and T. Misato, J. Bact., 104 (1970) 189.


207. (a) L.V. Vargha, Ber., 67 (1934) 1223.
    (b) K. Josephson, Ann., 472 (1929) 217.
    (c) H. Ohle, Ber., 62 (1929) 2885; 57 (1924) 403.
211. (a) H. Shechter, D.E. Ley and E.B. Robertson Jr.,
213. L.D. Hall, S.A. Black, K.N. Slessor and A.S. Tracey,
    (b) M.L. Wolfrom, P.J. Conigliaro and H.B. Bhat,
    (c) M.L. Wolfrom and P.J. Conigliaro, Carbohydr. Res.,
216. (a) H. Paulsen, Angew. Chem., 74 (1962) 901.