CONIGLIARO, Peter James, 1942—
SYNTHESIS OF NUCLEOSIDES OF 2-AMINO-2-
DEOXY SUGARS.
The Ohio State University, Ph.D., 1967
Chemistry, organic

University Microfilms, Inc., Ann Arbor, Michigan
SYNTHESIS OF NUCLEOSIDES OF 2-AMINO-2-DEOXY SUGARS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

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1967

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ACKNOWLEDGEMENTS

The author wishes to express his appreciation to Professor M. L. Wolfrom for suggesting the problems and for the advice given during the course of the research.

The author wishes to acknowledge the helpful advice given by Percy McWain, Dr. H. B. Bhat, Dr. E. J. Soltes, and Dr. M. W. Winkley during the research period.

This work was supported by Grants No. CA-03232-09, CA-03232-10, and CA-03232-11 from the Department of Health Education and Welfare, U. S. Public Health Service, National Institutes of Health, Bethesda, Md., to The Ohio State University Research Foundation (Projects 759 H, 759 I, and 759 J).
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PUBLICATIONS


FIELDS OF STUDY

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# CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>ii</td>
</tr>
<tr>
<td>VITA</td>
<td>iii</td>
</tr>
<tr>
<td>ILLUSTRATIONS</td>
<td>x</td>
</tr>
<tr>
<td>INTRODUCTION AND STATEMENT OF PROBLEM</td>
<td>1</td>
</tr>
<tr>
<td>HISTORICAL</td>
<td>11</td>
</tr>
</tbody>
</table>

**I. Natural Occurrence and Biological Importance of Nucleosides**

1. Nucleic Acids                                                        | 11   |
2. Nucleotide Anhydrides                                                | 23   |
3. Nucleoside Antibiotics                                               | 28   |
4. Other Naturally Occurring Nucleosides                                | 32   |

**II. General Synthetic Methods**

1. The Fischer-Helferich Method                                          | 34   |
2. The Hilbert-Johnson Method                                            | 36   |
3. The Mercuri Method                                                    | 37   |
4. The Trimethylsilyl Method                                             | 41   |
5. Methods Involving Direct Condensation Between Purines or Pyrimidines and Sugar Derivatives | 43   |
6. Stereospecificity in the Condensation Methods                         | 47   |
7. Methods Involving Interconversions of Nucleosides                    | 51   |
CONTENTS (Contd.)

8. Methods Involving Cyclization of N-Glycosyl Derivatives ............................................. 54

9. Nucleosides Synthesized by Various Methods ................................................................. 57

III. Synthesis of Nucleosides of 2-Amino-2-deoxy Sugars ............................................. 61

DISCUSSION OF RESULTS ................................................................. 76

I. Synthesis of Nucleosides of 2-Amino-2-deoxy-D-glucopyranose and 2-Amino-2-deoxy-D-galactopyranose ............................................................ 76

II. Synthesis of 2-Amino-1,1,2-trideoxy-1-ethythio-1-(1-thymyl)-D-glucose Aldehydrol ............................................................ 92

III. Synthesis of 1-(2-Amino-2-deoxy-β-D-glucofuranosyl)cytosine ............................................. 97

IV. Synthesis of 1-(2-Amino-2-deoxy-β-D-xylofuranosyl)thymine ............................................. 103

EXPERIMENTAL ................................................................. 108

I. Synthesis of Nucleosides of 2-Amino-2-deoxy-D-glucopyranose and 2-Amino-2-deoxy-D-galactopyranose ............................................................ 109

1. 1-[3,4,6-Tri-O-acetyl-2-N-[bis(phenoxy)phosphinyl]amino-2-deoxy-β-D-glucopyranosyl]cytosine (IIb) ............................................................ 109

2. 1-[2-N-[Bis(benzyloxy)phosphinyl]amino-2-deoxy-β-D-glucopyranosyl]cytosine (IIc) ............................................................ 110

3. 1-(2-amino-2-deoxy-β-D-glucopyranosyl)cytosine (III) ............................................................ 111

4. 2-Deoxy-2-trifluoroacetamido-D-glucose (V) ............................................................ 112
<table>
<thead>
<tr>
<th></th>
<th>CONTENTS (Contd.)</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>2-Deoxy-2-trifluoroacetamido-D-galactose (X)</td>
<td>113</td>
</tr>
<tr>
<td>6</td>
<td>1,3,4,6-Tetra-O-acetyl-2-deoxy-2-trifluoroacetamido-α- and -β-D-glucopyranose (VI and VII)</td>
<td>114</td>
</tr>
<tr>
<td>7</td>
<td>1,3,4,6-Tetra-O-acetyl-2-deoxy-2-trifluoroacetamido-α- and -β-D-galactopyranose (XI and XII)</td>
<td>116</td>
</tr>
<tr>
<td>8</td>
<td>Preparation of 3,4,6-Tri-O-acetyl-2-deoxy-2-trifluoroacetamido-α-D-glucopyranosyl Bromide (VII)</td>
<td>118</td>
</tr>
<tr>
<td>9</td>
<td>3,4,6-Tri-O-acetyl-2-deoxy-2-trifluoroacetamido-α-D-galactopyranosyl Bromide (XIII)</td>
<td>119</td>
</tr>
<tr>
<td>10</td>
<td>1-(3,4,6-Tri-O-acetyl-2-deoxy-2-trifluoroacetamido-β-D-glucopyranosyl)cytosine (XIV)</td>
<td>121</td>
</tr>
<tr>
<td>11</td>
<td>Deblocking of 1-(3,4,6-Tri-O-acetyl-2-deoxy-2-trifluoroacetamido-β-D-glucopyranosyl)cytosine (XIV)</td>
<td>123</td>
</tr>
<tr>
<td>12</td>
<td>6-Benzamido-9-(3,4,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-α- and -β-D-glucopyranosyl)purine (XVa and XVIIa)</td>
<td>123</td>
</tr>
<tr>
<td>13</td>
<td>9-(3,4,6-Tri-O-acetyl-2-deoxy-2-trifluoroacetamido-α-D-glucopyranosyl)-adenine Picrate (XVb)</td>
<td>126</td>
</tr>
<tr>
<td>14</td>
<td>9-(3,4,6-Tri-O-acetyl-2-deoxy-2-trifluoroacetamido-β-D-glucopyranosyl)-adenine Picrate (XVIIb)</td>
<td>126</td>
</tr>
<tr>
<td>15</td>
<td>9-(2-amino-2-deoxy-α-D-glucopyranosyl)-adenine (XVI)</td>
<td>127</td>
</tr>
<tr>
<td>16</td>
<td>9-(2-amino-2-deoxy-β-D-glucopyranosyl)-adenine (XVIII)</td>
<td>128</td>
</tr>
<tr>
<td>CONTENTS (Contd.)</td>
<td>Page</td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>17. 1- (3,4,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-β-D-galactopyranosyl)cytosine (XIX)</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>18. 1- (2-Amino-2-deoxy-β-D-galactopyranosyl)cytosine (XX)</td>
<td>129</td>
<td></td>
</tr>
<tr>
<td>19. 1- (3,4,6-Tri-O-acetyl-2-deoxy-2-trifluoroacetamido-β-D-galactopyranosyl)thymine (XXI)</td>
<td>130</td>
<td></td>
</tr>
<tr>
<td>20. 1- (2-Amino-2-deoxy-β-D-galactopyranosyl)thymine (XXII)</td>
<td>131</td>
<td></td>
</tr>
<tr>
<td>21. 6-Benzamido-9- (3,4,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-α- and β-D-galactopyranosyl)purine (XXIIIa and XXIVA)</td>
<td>132</td>
<td></td>
</tr>
<tr>
<td>22. 9- (3,4,6-Tri-O-acetyl-2-deoxy-2-trifluoroacetamido-α-D-galactopyranosyl)adenine picrate (XXIIIb)</td>
<td>134</td>
<td></td>
</tr>
<tr>
<td>23. 9- (3,4,6-Tri-O-acetyl-2-deoxy-2-trifluoroacetamido-β-D-galactopyranosyl)adenine picrate (XXIVb)</td>
<td>134</td>
<td></td>
</tr>
<tr>
<td>24. 9- (2-Amino-2-deoxy-α-D-galactopyranosyl)- adenine (XXIIIc)</td>
<td>135</td>
<td></td>
</tr>
<tr>
<td>25. 9- (2-Amino-2-deoxy-β-D-galactopyranosyl)- adenine (XXIVc)</td>
<td>136</td>
<td></td>
</tr>
<tr>
<td>26. 9- (2-Amino-2-deoxy-α-D-galactopyranosyl)- adenine Dihydrochloride (XXIIIId)</td>
<td>136</td>
<td></td>
</tr>
<tr>
<td>27. 9- (2-Amino-2-deoxy-β-D-galactopyranosyl)- adenine Dihydrochloride (XXIVd)</td>
<td>137</td>
<td></td>
</tr>
</tbody>
</table>

II. Synthesis of 2-Amino-1,1,2-trideoxy-1-ethylthio-1-(1-thyminyl)-D-glucose Aldehydrol | 138 |
| 1. 2-Deoxy-2-trifluoroacetamido-D-glucose Diethyl Dithioacetal (XXVI) | 138 |
## CONTENTS (Contd.)

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. 3,4,5,6-Tetra-O-acetyl-2-deoxy-2-trifluoroacetamido-D-glucose Diethyl Dithiocetal (XXVII)</td>
</tr>
<tr>
<td>3. 3,4,5,6-Tetra-O-acetyl-1,1,2-trideoxy-1-ethylthio-1-(1-thyminyl)-2-trifluoroacetamido-D-glucose Aldehyde, (+) and (−) Forms (XXIX and XXXI)</td>
</tr>
<tr>
<td>4. 2-Amino-1,1,2-trideoxy-1-ethylthio-1-(thyminyl)-D-glucose Aldehyde Hydrochloride, (+) Form (XXXb)</td>
</tr>
<tr>
<td>5. 2-Amino-1,1,2-trideoxy-1-ethylthio-1-(thyminyl)-D-glucose Aldehyde Hydrochloride (−) Form (XXXIIIb)</td>
</tr>
<tr>
<td>III. Synthesis of 1-(2-Amino-2-deoxy-β-D-glucofuranosyl)cytosine</td>
</tr>
<tr>
<td>1. Ethyl 2-Deoxy-1-thio-2-trifluoroacetamido-α-D-glucofuranoside (XXXIII)</td>
</tr>
<tr>
<td>2. Ethyl 3,5,6-Tri-O-acetyl-2-deoxy-1-thio-2-trifluoroacetamido-α-D-glucofuranoside (XXXVI)</td>
</tr>
<tr>
<td>3. 1-(3,5,6-Tri-O-acetyl-2-deoxy-2-trifluoroacetamido-β-D-glucofuranosyl)cytosine (XXXVIII)</td>
</tr>
<tr>
<td>4. 1-(2-Amino-2-deoxy-β-D-glucofuranosyl)cytosine Sulfate (XXXIXb)</td>
</tr>
<tr>
<td>5. Attempted Condensation of 3,5,6-Tri-O-acetyl-2-deoxy-2-(2,4-dinitroaminino)-D-glucofuranosyl Chloride with Bis-(trimethylsilyl)cytosine</td>
</tr>
<tr>
<td>IV. Synthesis of 1-(2-Amino-2-deoxy-β-D-xylofuranosyl)thymine</td>
</tr>
<tr>
<td>1. Ethyl 2-Deoxy-1-thio-2-trifluoroacetamido-α-D-xylofuranoside (XII)</td>
</tr>
</tbody>
</table>
CONTENTS (Contd.)

2. Ethyl 3,5-Di-Q-acetyl-2-deoxy-1-thio-2-
trifluoroacetamido-α-D-xylofuranoside
(XLI) ............................................................ 152

3. 1-(3,5-Di-Q-acetyl-2-deoxy-2-
trifluoroacetamido-β-D-xylo-
furanosyl)thymine (XLIII) ............................. 153

4. 1-(2-Amino-2-deoxy-β-D-xylofuranosyl)-
thymine hydrochloride (XLIVb) ....................... 154

SUMMARY ......................................................... 156

BIBLIOGRAPHY .................................................. 160
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>General Formulas for Nucleosides</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>The Major Ribonucleic Acid Nucleosides</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>The Major Deoxyribonucleic Acid Nucleosides</td>
<td>17</td>
</tr>
<tr>
<td>4</td>
<td>General Structure of Nucleic Acids</td>
<td>22</td>
</tr>
<tr>
<td>5</td>
<td>Adenosine 5'-Triphosphate</td>
<td>24</td>
</tr>
<tr>
<td>6</td>
<td>Cytidine Diphosphate Choline and Cytidine Diphosphate Ethanolamine</td>
<td>26</td>
</tr>
<tr>
<td>7</td>
<td>Uridine 5'-(α-D-Glucopyranosyl pyrophosphate), Uridine 5'-(α-D-Glucopyranosyluronic acid pyrophosphate), and Uridine 5'-(2-Acetanido-2-deoxy-α-D-glucopyranosyl pyrophosphate)</td>
<td>27</td>
</tr>
<tr>
<td>8</td>
<td>Puromycin</td>
<td>29</td>
</tr>
<tr>
<td>9</td>
<td>Nebularine and Cordycepin</td>
<td>31</td>
</tr>
<tr>
<td>10</td>
<td>Synthesis of 1-(2-Amino-2-deoxy-β-D-glucopyranosyl)cytosine using Bis(phenoxy)phosphinyl as N-Blocking group</td>
<td>78</td>
</tr>
<tr>
<td>12</td>
<td>Synthesis of Nucleosides of 2-Amino-2-deoxy-D-glucopyranose using Trifluoro-acetyl as N-blocking group</td>
<td>82</td>
</tr>
<tr>
<td>13</td>
<td>Synthesis of Nucleosides of 2-Amino-2-deoxy-D-galactopyranose</td>
<td>83</td>
</tr>
</tbody>
</table>
ILLUSTRATIONS (Contd.)

<table>
<thead>
<tr>
<th>Figure</th>
<th>Illustration Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>Synthesis of 2-Amino-1,1,2-trideoxy-1-ethylthio-1'(1-thyminyl)-D-glucose Aldehydrol</td>
<td>94</td>
</tr>
<tr>
<td>15</td>
<td>Synthesis of 1-(2-Amino-2-deoxy-β-D-glucofuranosyl)cytosine</td>
<td>98</td>
</tr>
<tr>
<td>16</td>
<td>Optical Rotatory Dispersion Spectrum of 1-(2-Amino-2-deoxy-β-D-glucofuranosyl)-cytosine Sulfate</td>
<td>103</td>
</tr>
<tr>
<td>17</td>
<td>Synthesis of 1-(2-Amino-2-deoxy-β-D-xylofuranosyl)thymine</td>
<td>105</td>
</tr>
<tr>
<td>18</td>
<td>Optical Rotatory Dispersion Spectrum of 1-(2-Amino-2-deoxy-β-D-xylofuranosyl)-thymine Hydrochloride</td>
<td>107</td>
</tr>
</tbody>
</table>

TABLE

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Amino Blocking Groups</td>
<td>62</td>
</tr>
</tbody>
</table>
Originally the term "nucleoside" was applied to the carbohydrate derivatives of purine and pyrimidine bases obtained by the hydrolysis of nucleic acids. It was first introduced by Levene and Jacobs (1)

(1) P. A. Levene and W. A. Jacobs, Ber., 42, 2474 (1909).

to describe the carbohydrate derivatives of purines isolated from the alkaline hydrolysates of yeast ribonucleic acid. The term was subsequently applied to all naturally occurring glycosyl derivatives of purines and pyrimidines and later to all such derivatives, whether naturally occurring or synthetically produced.

A nucleoside may be defined as a carbohydrate derivative of a purine or pyrimidine base in which the glycosyl carbon of the carbohydrate moiety is attached to the nitrogen atom at position 9 of the purine base or position 1 of the pyrimidine base (see figure 1).

\[
\text{\begin{align*}
\text{Purine Nucleoside} & \quad \text{Pyrimidine Nucleoside} \\
R & = \text{Glycosyl}
\end{align*}}
\]

Fig. 1. General Formulas for Nucleosides
term "aglycon" is usually used to describe the purine or pyrimidine moiety of a nucleoside.

Exceptions to this definition include two types of naturally occurring nucleosides: (1) those in which the point of attachment of the carbohydrate moiety to the aglycon is at a position other than N-9 (for purines) or N-1 (for pyrimidines), and (2) those in which the basic ring structure of the aglycon differs slightly from a true purine or pyrimidine. An example of the first type of nucleoside is 7-β-D-ribofuranosyladenine, obtained by degradation of pseudovitamin B₁₂ (2).

(2) W. Friedrich and K. Bernhauer, Angew. Chem., 68, 580 (1956); Ber., 82, 2507 (1956).

An example of the second type of nucleoside is tubercidin (4-amino-7-β-D-ribofuranosylpyrrolo[2,3-d] pyrimidine, a nucleoside of 7-deaza-adenine) isolated by Suzuki and Maruma (3). Such compounds are often referred to as "pseudonucleosides". Very few of these types of naturally occurring compounds have been isolated.

Most naturally occurring pyrimidine nucleosides contain a carbonyl group at position 2 of the aglycon and a substituent (usually an amino or hydroxyl group) at position 4 of the aglycon, while most naturally occurring purine nucleosides contain substituents (usually amino or hydroxyl groups) at positions 2 and/or 6 of the aglycon. Such substituents give rise to tautomeric equilibria which is illustrated here.
for the case of α-uracil nucleoside.

\[
\begin{align*}
\text{OH} & \quad \text{O} \\
\text{N} & \quad \text{N}' \\
\text{H} & \quad \text{R} = \text{Glycosyl}
\end{align*}
\]

\[\text{H} \quad \text{O}' \quad \text{R}\]

In this dissertation, nucleosides are named as glycosyl derivatives of purines or pyrimidines, with the exception of acyclic sugar nucleoside analogs and certain naturally occurring nucleosides which have commonly accepted trivial names. When using trivial names, the primed numerals refer to positions in the sugar moiety. The numbering system used for purines and pyrimidines (see figure 1) is in accord with the usage of Chemical Abstracts (4).


Major interest in nucleosides began with the discoveries by Levene and co-workers that they were components of the biologically important ribonucleic (1, 5) and deoxyribonucleic (6, 7) acids. As

(5) P. A. Levene and W. A. Jacobs, Ber., 43, 3150 (1910).

(6) P. A. Levene and E. S. London, J. Biol. Chem., 81, 711 (1929).

(7) P. A. Levene and E. S. London, ibid., 83, 793 (1929).

components of such entities as chromosomes and viruses and as agents
in cell growth, these nucleic acids play important roles in biological life processes such as cell reproduction and transmission of genetic information. It is now known that nucleosides, in the form of phosphate esters (nucleotides), constitute the polymeric units of nucleic acids of all cells. The discovery of naturally occurring nucleosides in entities other than nucleic acids has further stimulated interest in nucleosides. The most important of these non-nucleic acid nucleosides are those which form components of nucleotide anhydrides (8) and


those which possess antibiotic properties (9).


Initially, efforts to develop chemical methods of synthesis of nucleosides were concentrated primarily on the eventual synthesis of naturally occurring nucleosides, especially those found in nucleic acids. This work was undertaken to verify the structures deduced for these nucleosides by synthesizing them from their component fragments and to provide methods of synthesizing naturally occurring nucleosides which were difficult to isolate from their natural sources. More recently, the demand for antibiotic nucleosides, as well as the possible utility of "abnormal" nucleosides as chemotherapeutic agents against neoplastic diseases, has intensified efforts to develop methods of
synthesizing a wide variety of nucleosides which are not naturally occurring as well as naturally occurring nucleosides. The observations that certain purine and pyrimidine bases themselves, such as 5-fluorouracil (10), and 6-mercaptopurine (11), are effective in the con-


trol of certain neoplastic diseases, such as cancer, has further stim-
ulated these efforts, especially with regard to the synthesis and in-
vestigation of nucleosides containing these "active" bases.

Special interest in nucleosides of amino sugars began with the discovery that the antibiotic puromycin was a nucleoside derivative of 3-amino-3-deoxy-D-ribose (12-14). Subsequent observations that several


other amino sugar nucleoside derivatives exhibited antibacterial and antitumor properties (13, 15-18) has stimulated much interest in the


The first chemical synthesis of a wide variety of amino sugar nucleosides. Chemical synthesis of a wide variety of amino sugar nucleosides.

The first chemical synthesis of a nucleoside was achieved by Fischer and Helferich (19). Condensation of tetra-O-acetyl-a-D-glucopyranosyl bromide with the silver salt of 2,6-dichloropurine (theophylline), and subsequent deacetylation of the product, gave 2,6-dichloro-7-β-D-glucopyranosylpurine. Since then, a variety of synthetic methods have been developed for the synthesis of purine and pyrimidine nucleosides.

The first chemical synthesis of a naturally occurring nucleoside was achieved by Todd and co-workers in 1947 (20). Condensation of

(19) E. Fischer and B. Helferich, Ber., 47, 210 (1914).

crude tri-β-acetyl-D-ribofuranosyl bromide with 2,4-diethoxyypyrimidine followed by treatment of the crude product with alcoholic ammonia afforded 1-β-D-ribofuranosylcytosine (cytidine). All of the principal naturally occurring nucleic acid nucleosides have now been synthesized chemically.

The wide variety of synthetic methods available in recent times has also made possible the synthesis of numerous nucleosides with interesting variations in both the carbohydrate and aglycon moiety. For example, nucleosides have been synthesized containing disaccharides (21, 22), ketoses (23, 24), and unsaturated sugars (25) as the carbohydrate moieties.

(22) M. L. Wolfson, P. McWain, and A. Thompson, ibid., 82, 4353 (1960).

The recent interest in amino sugar nucleosides as possible antitumor or antibacterial agents has led to the synthesis of a large number of nucleosides of this type. The majority of these are nucleosides of 3-amino-2-deoxy and 5-amino-5-deoxy sugars. Special problems have
been encountered in the synthesis of nucleosides of 2-amino-2-deoxy sugars, however, and relatively few nucleosides of this type have been synthesized. The only 2-amino-2-deoxy sugar for which a significant number of nucleosides have been synthesized is 2-amino-2-deoxy-D-glucose. The major source of difficulty encountered in the synthesis of nucleosides of 2-amino-2-deoxy sugars has been the selection of an effective amino blocking group. In 1954 Baker and co-workers (26)


achieved the first synthesis of a nucleoside of a 2-amino-2-deoxy sugar, 9-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-6-dimethylamino-2-methylthioadenine. It was not until five years later, however, that the removal of an N-blocking group was achieved to give a completely deblocked nucleoside of this type (27, 28).


To this date, the syntheses of only nine completely deblocked nucleosides of 2-amino-2-deoxy sugars have been reported, not counting anomeric forms. Of these, five are nucleosides of 2-amino-2-deoxy-D-glucose: 1-(2-amino-2-deoxy-β-D-glucopyranosyl)cytosine (27), 1-(2-amino-2-deoxy-β-D-glucopyranosyl)uracil (27), 1-(2-amino-2-deoxy-β-D-glucopyranosyl)thymine (29, 30), 9-(2-amino-2-deoxy-α- and -β-D-


Glucopyranosyladenine (31), and 9-(2-amino-2-deoxy-α- and -β-D-


Glucofuranosyladenine (32); one is a nucleoside of 2-amino-2,3-


dideoxy-D-erythro-pentose: 9-(2-amino-2,3-dideoxy-β-D-erythro-pento-


Ribose: 9-(2-amino-2-deoxy-β-D-ribofuranosyl)-6-dimethylamino-purine (34) and 9-(2-amino-2-deoxy-α- and -β-D-ribofuranosyl)adenine (32, 35);


and the remaining one is a nucleoside of 2-amino-2-deoxy-D-allose: 9-(2-amino-2-deoxy-β-D-allopyranosyl)-6-dimethylaminopurine (2S). The limited number of nucleosides of 2-amino-2-deoxy sugars which have been synthesized was a principal factor in the undertaking of the work described herein.

The work described in this dissertation is concerned with the synthesis of nucleosides of several different 2-amino-2-deoxy sugars. The purpose of this work was to synthesize nucleosides which may have value as antibacterial and antitumor agents (especially as cancer chemotherapeutic agents) and to develop general procedures for the synthesis of various types of nucleosides of 2-amino-2-deoxy sugars. Since it was considered desirable to synthesize nucleosides of several different types of amino sugars, it was decided to undertake the synthesis of nucleosides of 2-amino-2-deoxy sugars wherein the sugar moiety is a hexopyranose, an acyclic aldehydo-hexose, a hexofuranose, and a pentofuranose. The following problems were undertaken:

1. The synthesis of nucleosides of the pyranose form of 2-amino-2-deoxy-D-galactose.

2. The synthesis of a nucleoside of an acyclic form of 2-amino-2-deoxy-D-glucose.

3. The synthesis of a nucleoside of the furanose form of 2-amino-2-deoxy-D-glucose.

4. The synthesis of a nucleoside of the furanose form of 2-amino-2-deoxy-D-xylose.
I. Natural Occurrence and Biological Importance of Nucleosides

The first nucleoside to be discovered was guanosine (first called "vernine") which was isolated by Schulze and Bosshard in 1885 (36).

(36) E. Schulze and E. Bosshard, Z. physiol. Chem., 2, 443 (1885); 10, 80 (1886).

In subsequent years, numerous nucleosides have been isolated from various natural sources. Until 1927, the only known natural sources of nucleosides were nucleic acids and the early work on nucleosides was stimulated mainly by interest in them as components of these nucleic acids. In the past fifty years, however, a large number of nucleosides have been isolated from natural sources other than nucleic acids. The two most important classes of non-nucleic acid nucleoside derivatives are nucleotide anhydrides and nucleoside antibiotics. A significant number of naturally occurring nucleosides have been found in uncombined form.

1. Nucleic Acids

Nucleic acids are found as components of the cytoplasm and nuclei of living cells. It is generally recognized that the nucleic acids are of fundamental importance in controlling metabolism, reproduction,
growth, and transmission of genetic information of living systems.

The first isolation of a nucleic acid is credited to Miescher (37).


Working in the laboratory of Hoppe-Seyler from 1868 to 1869, he was able to obtain nuclear material by digesting pus cells with hydrochloric acid. From this material he isolated an acidic substance which contained a high percentage of phosphorus and which he named "nuclein". Since the time of this first isolation of a nucleic acid, a large number of nucleic acids have been isolated from various sources. The increasing recognition of the biological importance of nucleic acids, as well as their isolation from numerous natural sources, stimulated a great deal of effort to determine their structures.

The first information concerning the structures of nucleic acids was gained through hydrolytic studies. In initial studies, vigorous acid hydrolysis of nucleic acids from various sources produced phosphoric acid, five major nitrogenous bases, and certain carbohydrates and their degradation products.

All of the nitrogenous bases were identified by 1903 principally through the efforts of Kossel and co-workers. Two purine bases, adenine (38) and guanine (39), were the first to be identified.

(38) A. Kossel, Ber., 18, 1928 (1885); Z. physiol. Chem., 10, 248 (1886).

(39) A. Kossel, ibid., 8, 404 (1883-1884).
Their correct structures, however, were not deduced until several years later by Fischer (40). The three remaining bases were subsequently identified as the pyrimidine bases thymine (41), cytosine (42), and uracil (43).

(40) E. Fischer, Ber., 20, 2226 (1897).

(41) A. Kossel and A. Neumann, ibid., 26, 2753 (1893).

(42) A. Kossel and A. Neumann, ibid., 27, 2215 (1894).

(43) A. Ascoli, Z. physiol. Chem., Hoppe-Seyler's, 21, 161 (1900-01).

The identification of the carbohydrate components of nucleic acids showed that there were two principal types of nucleic acids: one which contained D-ribose as the carbohydrate component, and subsequently designated ribonucleic acid (RNA), and another which contained 2-deoxy-D-erythro-pentose ("deoxyribose") as the carbohydrate component, and subsequently designated deoxyribonucleic acid (DNA). The identification of D-ribose as the carbohydrate component of ribonucleic acids was achieved by Levene and Jacobs (44-46). After first succeeding in isolating the sugar in crystalline form, they found that it was not

(44) P. A. Levene and W. A. Jacobs, Ber., 41, 2703 (1908).

(45) P. A. Levene and W. A. Jacobs, ibid., 42, 1198 (1909).

(46) P. A. Levene and W. A. Jacobs, ibid., 44, 746 (1911).
identical to any sugar which had been characterized up to that time. Since its optical rotation was equal in magnitude but opposite in sign to synthetic L-ribose, with all other physical properties being identical, they concluded that the sugar was D-ribose. This conclusion was later verified when D-ribose was synthesized (47) and shown to be identical with the natural D-ribose of Levene and Jacobs.

The identification of 2-deoxy-D-erythro-pentose as the carbohydrate component of deoxyribonucleic acids proved to be more difficult since this sugar decomposed during chemical hydrolysis of the nucleic acid. The isolation of a deoxyribonucleic acid nucleoside, 9-(2-deoxy-β-D-erythro-pentofuranosyl)guanine (2'‑deoxyguanosine), by Levene and London in 1929 (6, 7) finally made possible the isolation of this sugar. By subjecting 2'-deoxyguanosine to very mild acid hydrolysis, 2-deoxy-D-erythro-pentose was obtained in crystalline form (6, 7). The identification of this sugar was made by comparison with synthetic 2-deoxy-D-threo-pentose and 2-deoxy-L-erythro-pentose (48).


After identification of the principal components of nucleic acids, the next problem was to determine how these components are linked. The fact that the carbohydrate components and nitrogenous bases are present as nucleosides was demonstrated by the actual isolation of the compo-
nent nucleosides of nucleic acids. The ribonucleic acid nucleosides were the first to be isolated. Four major ribonucleosides were obtained: 1-β-D-ribofuranosylcytosine (cytidine), 1-β-D-ribofuranosyluracil (uridine), 9-β-D-ribofuranosyladenine (adenosine) and 9-β-D-ribofuranosylguanine (guanosine). The structures of these nucleosides are given in figure 2. These four nucleosides are still recognized as the

Fig. 2. The Major Ribonucleic Acid Nucleosides
major nucleosidic components of the vast majority of ribonucleic acids. Adenosine and guanosine were isolated by Levene and Jacobs (1, 49) from yeast ribonucleic acid by hydrolysis under mildly basic conditions. They subsequently obtained cytidine and uridine (50) by acid hydrolysis of yeast nucleic acid followed by treatment of the hydrolysate with aqueous ammonia.

The isolation of nucleosides from deoxyribonucleic acids presented special problems due to the relative instability of the 2-deoxy-D-erythro-pentose moiety. Thus, attempts to obtain nucleosides by acid or basic hydrolysis of deoxyribonucleic acids resulted in the decomposition of the sugar moiety. Nucleosides were finally obtained from deoxyribonucleic acids by enzymic hydrolysis employing intestinal juices from animals. Four major nucleosides were obtained from deoxyribonucleic acids: 1-(2-deoxy-D-erythro-pentofuranosyl)cytosine (2'-deoxycytidine), 1-(2-deoxy-D-erythro-pentofuranosyl)thymine (thymidine), 9-(2-deoxy-D-erythro-pentofuranosyl)adenine (2'-deoxyadenosine), and 9-(2-deoxy-D-erythro-pentofuranosyl)guanine (2'-deoxyguanosine. The structures of these nucleosides are given in figure 3. These four nucleosides are still recognized as the major nucleosidic components of the majority of deoxyribonucleic acids. The first isolation of deoxyribonucleosides was accomplished by Levene and London in 1929. They succeeded in hydrolyzing a deoxyribonucleic acid
Fig. 3. The Major Deoxyribonucleic Acid Nucleosides

by passing an aqueous solution of it through a segment of the gastro-intestinal tract of a dog and obtained 2'-deoxyguanosine (6, 7), 2'-deoxycytidine (7), and thymidine (7). The fourth major deoxyribonucleic acid nucleoside, 2'-deoxyadenosine, was isolated by Klein in 1933 (51).
After the isolation of the major nucleosides from nucleic acids, the structures of the nucleosides, their mode of linkage by the phosphate portion of the nucleic acid, and their sequences in various nucleic acids remained to be determined. The determination of the structures of the nucleosides involved the following: (1) the nature of the sugar and aglycon moieties, (2) the points of linkage at the aglycon rings, (3) the ring structure of the sugars, and (4) the configuration at the glycosidic carbon of the sugars.

The nature of the sugar and aglycon moieties of the various nucleosides was established by hydrolysis of the nucleosides into their sugar and aglycon components and subsequent isolation and characterization of these components. The purine nucleosides could be hydrolyzed by acid, but the pyrimidine nucleosides required special hydrolytic procedures. Since D-ribose and 2-deoxy-D-erythro-pentose had been obtained from ribonucleic acids and deoxyribonucleic acids respectively, it had been assumed that these sugars were the components of the ribonucleosides and deoxyribonucleosides, respectively. The identification of the sugar component of each nucleoside confirmed this assumption.

The points of linkage at the purine and pyrimidine aglycons were shown to be at positions 9 and 1 respectively by spectroscopic evidence (52, 53), certain substitution reactions at the aglycon moieties (54, 55).

(51) W. Klein, Z. physiol. Chem., 224, 244, 252 (1934).

55), and by synthesis of various nucleosides by cyclization of N-


glycosyl derivatives of certain acyclic compounds (53, 56).


The ring structures of the sugars were deduced from certain chemical studies including tritylation experiments (52, 57), degrada-

(57) P. A. Levene and R. S. Tipson, J. Biol. Chem., 106, 113 (1934); 109, 623 (1935); 121, 131 (1937); Science, 81, 98 (1935).

tion of methylated nucleosides (52, 58), complex formation with

(58) P. A. Levene and R. S. Tipson, J. Biol. Chem., 92, 623 (1931); 94, 809 (1932); 97, 491 (1932); 101, 529 (1933); Science, 74, 521 (1931).

borate (59) (for deoxyribonucleosides), and periodate oxidation (60, 61).


The configurations at the glycosidic centers of the sugars were determined by periodate oxidation (61), X-ray crystallographic studies (62), and by the formation of certain anhydronucleosides such as I and II below.


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![Diagram](image)

I (63)  

II (64)

The formation of such anhydronucleosides could only take place if the configuration at the glycosidic center is $\beta$-D.

The nature of the phosphate linkages have been deduced from various investigations which will not be discussed here. For reviews of these investigations see Barker (65) and Michelson (66).

Today it is generally recognized that nucleic acids are polymeric substances consisting of nucleoside units linked alternately at positions 3 and 5 of the carbohydrate moiety by phosphate ester linkages (see figure 4). Since phosphate esters of nucleosides are nucleotides, nucleic acids may also be regarded as polynucleotides.

Both ribonucleic acids and deoxyribonucleic acids are present in all types of cells, both plant and animal. The main biological distinction is that ribonucleic acids are present principally in the cytoplasm of cells while deoxyribonucleic acids are present principally in the nuclei of cells (67). Although the eight nucleosides shown in figures 2 and 3 are the major constituents of nucleic acids, several
Ribonucleic Acids:  \( R = \text{OH}; \ B = \text{Adenine, Guanine, Cytosine, or Uracil} \)

Deoxyribonucleic Acids:  \( R = \text{H}; \ B = \text{Adenine, Guanine, Cytosine, or Thymine} \)

Fig. 4. General Structure of Nucleic Acids

Other nucleosides have been found as minor components of nucleic acids. A few nucleic acids have also been found to contain nucleosides other than those given in figures 2 and 3 as major components. For example, small amounts of \( 9-(2\text{-deoxy-\(\beta\text{-D-erythro-pentofuranosyl}\})-6\text{-methylaminopurine} \) and \( 9-(2\text{-deoxy-\(\beta\text{-D-erythro-pentofuranosyl} \))-6\text{-dimethylaminopurine} \)
have been identified in deoxyribonucleic acids from various bacteria and bacteriophage (68) and 1-(2-deoxy-β-D-erythro-pentofuranosyl)-


5-hydroxymethylcytosine occurs in place of 1-(2-deoxy-β-D-erythro-
pentofuranosyl)cytosine as one of the four major component nucleosides in Escherichia coli T even numbered bacteriophage deoxyribonucleic acids (69).

(69) G. R. Wyatt and S. S. Cohen, ibid., 55, 774 (1953).

Since the sequence of nucleosides varies with each nucleic acid, its determination is a separate problem for each individual nucleic acid. Experimental determination of this sequence is a major problem of the present day.

2. Nucleotide Anhydrides

Another important group of nucleoside derivatives are the nucleotide anhydrides. Included in this group of substances are a variety of compounds which are key intermediates in various metabolic processes and which are essential to various energy transfer processes in biological systems. With few exceptions, the biologically important nucleotide anhydrides are 5'-nucleotide anhydrides, that is, anhydrides of nucleoside 5'-phosphates and another acid which may be pyrophosphoric acid, phosphoric acid or monoesters thereof, carboxylic acids, amino acids and peptides, or sulfuric acid. These substances are sometimes loosely designated "nucleotide coenzymes" but this term is not widely
used today.

In recent times, knowledge of the biological significance of the nucleotide anhydrides has expanded rapidly and the biochemistry of such compounds has been quite extensively determined. For example, they have been found to be important intermediates in various electron transport and oxidation-reduction processes, biological transphosphorylation, and transfer of sugars, carboxylic acids, amino acids, sulfates, and nucleotides. A large number of nucleotide anhydrides have been isolated and investigated but only a brief description of a few of the more important ones will be given here.

Probably the most important nucleotide anhydride is adenosine 5'-triphosphate (ATP) which is a key intermediate in a large number of energy transfer processes in living organisms. It was first isolated in 1929 from muscle extracts (70) and has been found (71, 72) to have the structure shown in figure 5.

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(70) C. H. Fiske and Y. Subbarow, Science, 70, 381 (1929).
(71) K. Lohmann, Biochem. Z., 232, 460 (1931); 242, 381 (1932).

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Fig. 5. Adenosine 5'-Triphosphate (ATP)
Other important nucleotide anhydrides containing adenosine as the nucleoside component include flavine adenine dinucleotide (73), nicotinamide adenine dinucleotide (74, 75), and coenzyme A (76).


Two important nucleotide anhydrides containing cytidine as the nucleoside component are cytidine diphosphate choline and cytidine diphosphate ethanolamine (77) which are intermediates in the synthesis of phospholipids. The structures of these two compounds are represented in figure 6.


An important class of nucleotide anhydrides are the glycosyl nucleotides. These substances are anhydrides of 5'—nucleotides and glycosyl esters of phosphoric acid, that is, they are substances which consist of a nucleoside bound to a glycosyl moiety through a pyrophosphate linkage at the 5' position. These substances are key intermediates in various metabolic processes involving sugar transfer. The
most important group of glycosyl nucleotides are those which contain uridine as the nucleoside component. A significant number of these compounds have been isolated and shown to be of considerable importance in such processes as sugar metabolism, biosynthesis of polysaccharides, and cell wall synthesis. The structures of three of these, uridine 5'-(-D-glucopyranosyl pyrophosphate) (the most widely distributed of the glycosyl nucleotides) (78, 79), uridine 5'-(-D-

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(78) I. Liberman, ibid., 223, 327 (1956).
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and uridine 5'-(-2-

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acetamido-2-deoxy-α-D-glucopyranosyl pyrophosphate) (81) are represented in figure 7.


Other important glycosyl nucleotides include such compounds as guanosine 5'-α-D-mannopyranosyl pyrophosphate and thymidine 5'-α-D-rhamnopyranosyl pyrophosphate) (82, 83). A large number and diversity


of glycosyl nucleotides have been tabulated and their chemistry discussed by Neufeld and Hassid (79).

3. Nucleoside Antibiotics

A third important class of nucleoside derivatives are the nucleoside antibiotics. Unlike the nucleic acids or nucleotide anhydrides, there is no structural feature common to all the members of this group other than the fact that they are nucleoside derivatives. These substances are classified as a group because of their antibiotic properties.

Derivatives of amino sugar nucleosides are especially prominent among the nucleoside antibiotics. Interest in amino sugar nucleosides has stemmed primarily from discoveries of the antibiotic properties exhibited by several amino sugar nucleoside derivatives, especially with regard to antitumor properties. The potential usefulness of amino sugar nucleoside derivatives as possible antitumor and anticancer agents was, in fact, the major factor which prompted the undertaking of the work described in this dissertation.

The first antibiotic substance containing an amino sugar nucleoside to be discovered was puromycin which was first isolated from streptomycetes alboniger (12). It has been found to be active against Trypanosoma equiperdum and other protozoa and inhibit the growth of mammary adenocarcinoma in mice (12). By degradation studies (12, 13) puromycin was shown to have the structure represented in figure 8. This structure was also confirmed by the total synthesis of puromycin (14).

The amicetin group antibiotics (amicetin, bamicetin, and plicacetin) constitute an important group of nucleoside type antibiotics containing amino sugars. They have been isolated from Streptomyces
Fig. 8. Puromycin

Vinacengrappus and Streptomyces fasciculatic (15) and have been found to exhibit antitubercular activity (84–87). These substances all contain an N-acylated cytosine linked to a 3-amino-3,4-dideoxyglycopyranose moiety through 1,4-dimethyltetrahydrofuran (16, 87). Because of this type of linkage they are not usually considered true amino sugar nucleosides, however.

Among other amino sugar nucleosides exhibiting antibiotic properties are 9-(3-amino-2,3-dideoxy-β-D-erythro-pentofuranosyl)adenine

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which has been found to be active against ascitic tumors in mice (17) and 9-[(3-amino-3-deoxy-β-D-ribofuranosyl)-6-dimethylamino]-purine which has been found to be more active than puromycin against mammary adenocarcinoma in mice (13).

Amino sugar nucleoside derivatives constitute only a part of the group of nucleoside antibiotics. Other members of this group contain nucleosides which consist of a wide variety of sugars and aglycons. Two additional important nucleoside antibiotics are cordycepin, which has been isolated from the mould Cordyceps militaris and found to be active against B. subtilis and avian tubercle bacillus (88), and


nebularine, which has been isolated from the mushroom Agaricus nebularis (89) and been found to exhibit activity against muco bacteria and


mouse Sarcoma 180 (90). The structures of cordycepin (91) and nebularine (92) have been determined and are represented in figure 9.


Other important nucleoside antibiotics include psicofurrane (an adenine nucleoside of piscose) (93), which shows antibacterial and antitumor activity, nucleocidin (94), a Streptomyces product which shows antitrypanosomal activity, toyocamycin (a cyano derivative of 7-deaza-adenosine) (95), which is active against Mycobacterium tuberculosis,
4. Other Naturally Occurring Nucleosides

A number of nucleosides, which fulfill a variety of biological functions but do not fall under any single classification, have been isolated from a wide variety of natural sources such as yeast, beef extracts, various moulds, sponges, and liver. Some of the more important of these include crotonoside (9-β-D-ribofuranosylisoguanine) (97),


isolated from the croton bean, spongosine (98), isolated from sponges,


orotidine (1-β-D-ribofuranosyluracil-6-carboxylic acid) (99), isolated

from bread mould and milk, and uric acid ribonucleoside (3-D-ribo-syluric acid) (100), isolated from beef blood.


II. General Synthetic Methods

Chemical methods of nucleoside synthesis can be divided into three general categories:

(1) Methods involving condensation between a glycosyl derivative (usually a halide) and a purine or pyrimidine derivative:

\[ RX + BY \rightarrow RB + XY \]

where \( R \) represents the glycosyl moiety and \( B \) represents the purine or pyrimidine moiety.

(2) Methods involving interconversion of a nucleoside by alteration of the sugar or aglycon moiety of the nucleoside:

\[ RB \rightarrow R' B \quad \text{or} \quad RB \rightarrow RB' \]

where \( R \) and \( R' \) represent the sugar moiety and \( B \) and \( B' \) represent the aglycon.

(3) Methods involving cyclization of an N-glycosyl derivative of an acyclic compound to give a nucleoside:

\[ R-X \rightarrow RB \]

where \( R \) represents a glycosyl moiety, \( X \) represents an acyclic fragment and \( B \) represents a purine or pyrimidine aglycon.

Several different methods of the first type will be described here,
but the latter two types of methods, since they do not apply to the present work, will be only briefly discussed.

1. The Fischer-Helferich Method

The first method for the synthesis of nucleosides was developed by Fischer and Helferich (19). Their method consisted of condensation of a per-α-acylglycosyl halide with a silver salt of an appropriate purine. Their first synthesis of a nucleoside by this method yielded only a 7-glycosylpurine. Tetra-α-acetyl-α-D-glucopyranosyl bromide (III) was condensed with the silver salt of 2,6-dichloropurine (theophylline) (IV) to give, after deacetylation of the initial product, 7-β-D-glucopyranosyltheophylline (V).

\[ \text{III} + \text{IV} \xrightarrow{\text{Deacetylation}} \text{V} \]

By using other purine derivatives, however, 9-glycosylpurines were obtained. For example, condensation of tetra-α-acetyl-α-D-glucopyranosyl bromide with the silver salt of 2,8-dichloroadenine gave, after deacetylation of the initial product, 2,8-dichloro-9-β-D-glucopyranosyladenine (19). By suitable reactions involving replacement of the chlorine atoms and diazatocation of the amino group, this nucleoside
was converted to the corresponding adenine and guanine nucleosides.

In general, the Fischer-Helferich procedure leads to 9-glycosylpurines, with the important exception of theophylline nucleosides. If an acyl or other participating group is present at C-2 of the sugar, the exclusive or predominant product is the nucleoside bearing a trans relationship between the aglycon and the substituent at C-2 of the sugar ("trans-nucleoside"). This has been formulated as the "trans rule" by Tipson (101) and extended by Baker (26, 102).


The Fischer-Helferich procedure has been used for the first synthesis of a naturally occurring purine nucleoside, adenosine (103).


The original procedure is somewhat limited in that low yields are generally obtained and that it cannot be applied to purines containing an amino group basic enough to react with the glycosyl halide employed. These difficulties, however, have been largely overcome by suitable modifications and this procedure (in modified form) is still a widely used method for the synthesis of purine nucleosides.

Attempts to apply the Fischer-Helferich procedure to the synthesis of pyrimidine nucleosides were unsuccessful. In attempting to prepare
nucleosides using silver salts of various substituted pyrimidines, Fischer (19, 104), and later Levene and Sobotka (105), isolated only substances which reduced Fehling's solution and were easily hydrolyzed to sugars and aglycons by dilute acid (properties not common to pyrimidine nucleosides). These substances were concluded to be glycosides in which the sugar is linked to position 2 of the pyrimidine through an oxygen atom.

2. The Hilbert–Johnson Method

The first general method for synthesizing pyrimidine nucleosides was developed by Hilbert and Johnson (106). This method consisted of condensation of a 2,4-dialkoxypyrimidine with a per-O-acylglycosyl halide to yield a 4-alkoxy-1-glycosylpyrimidine. By treatment with acid or ammonia the alkoxy group could then be replaced with hydroxyl or amino, respectively, giving a uracil or cytosine nucleoside (106–108).

The uracil and cytosine nucleosides of D-glucose were the first to
be synthesized by this procedure (106-108). Condensation of tetra-\(\text{O-}
\)acetyl-\(\alpha\)-D-glucopyranosyl bromide (III) with 2,4-diethoxypyrimidine (VI) gave 4-ethoxy-(3,4,6-tri-\(\text{O-}
\)acetyl-\(\beta\)-D-glucopyranosyl)pyrimidine (VIII), presumably involving the intermediate VII.

III  +  VI  \rightarrow  VII  \rightarrow  VIII

Treatment of VIII with methanolic hydrogen chloride gave 1-\(\beta\)-D-glucopyranosyluracil, while treatment of VIII with methanolic ammonia gave 1-\(\beta\)-D-glucopyranosylcytosine.

Various substituted pyrimidines have since been employed in the Hilbert-Johnson procedure to yield a variety of nucleosides. With sugars containing participating groups at C-2, the trans-nucleosides are formed exclusively or predominantly. The first synthesis of a naturally occurring nucleoside, cytidine, was accomplished using the Hilbert-Johnson procedure (20).

3. The Mercuri Method

A significant modification of the Fischer-Helferich procedure for the preparation of purine nucleosides was made by Davoll and Lowy (109).
who employed chloromercuri derivatives of purines in place of silver salts of purines in the reaction with poly-α-acyl glycosyl halides. As an additional modification, they blocked any basic amino group on the purine ring by acylation. By these modifications, not only were the yields of nucleosides greatly improved, but many more purine derivatives could be employed for the condensation reaction.

As an example of an early application of this mercuri procedure may be cited the reaction of tri-α-acetyl-D-ribofuranosyl chloride (IX) with 6-benzamido-9-chloromercipurine (X) to give the blocked nucleoside derivative (XI) which was then deacylated to give adenosine in 27% overall yield based on the ribosyl halide (IX) (109).

Although earlier approaches to direct coupling of metal-pyrimidines and sugar halides to form nucleosides failed, Fox and co-workers (110)
were able to extend the mercuri method to the synthesis of pyrimidine nucleosides by using mercuri derivatives of pyrimidines. These derivatives contain the pyrimidine base and mercury in a 1:1 or 2:1 molar ratio. The precise structures of the mercuri derivatives employed are unknown, however. In the case of cytosine derivatives, the amino group had to be acylated. An example of an early application of the mercuri method to pyrimidine nucleoside synthesis is the synthesis of 1-β-D-ribofuranosylthymine (XIV) (110). Condensation of tri-O-benzyl-D-ribofuranosyl chloride (XII) with dithyminylmercury (XIII) and subsequent debenzoylation of the product with alcoholic ammonia gave 1-β-D-ribofuranosylthymine (XIV).

\[ \text{CH}_3\text{OH} \]  
\[ \text{CH}_2\text{O}_{\text{Bz}} \]  
\[ \text{OH} \]  
\[ \text{NH} \]  
\[ \text{CH}_3 \]  
\[ \text{CO} \]  
\[ \text{NH} \]  
\[ \text{CO} \]  
\[ \text{OH} \]  
\[ \text{OH} \]  
\[ \text{XII} \]  
\[ \text{XIII} \]  
\[ \text{XIV} \]

In the synthesis of purine nucleosides, 9-glycosylpurines are nearly always obtained with a few exceptions (111, 112). In the case


(111) B. R. Baker, J. P. Joseph, R. E. Schaub, and J. H. Williams,
of the synthesis of pyrimidine nucleosides, the glycosylpyrimidines are always formed except in a few isolated cases in which O'-glycosides are formed (113, 114). It was found that these glycosides and other glycosides formed by condensation of silver salts of pyrimidines with acetylated glycosyl halides (115) rearranged to nucleosides (1-glycosyl-
pyrimidines) by treatment with various mercury salts (113-115).

Ulbricht (116) proposed that in pyrimidine nucleoside synthesis, the glycoside is initially formed but rearranges to the nucleoside under the reaction conditions in the presence of the mercury salt. In both purine and pyrimidine nucleoside synthesis by the mercuri method, the trans rule (101, 102) is followed, that is, the trans-nucleoside is formed exclusively or predominantly whenever there is a participating
group at C-2 of the sugar. The mercuri method has been more extensively employed for nucleoside synthesis (especially for the synthesis of purine nucleosides) than any other method. A large number and diversity of nucleosides have been synthesized using this method.

4. The Trimethylsilyl Method

A recently developed method of nucleoside synthesis involves the condensation of a trimethylsilyl substituted purine or pyrimidine with an acetylated glycosyl halide to yield a blocked nucleoside. This method was first used by Birkofeर (117) for the synthesis of 3-β-D-


rribofuranosyl uric acid. Condensation of tetrakis(trimethylsilyl)uric acid with tri-β-benzoyl-D-ribofuranosyl bromide in the presence of silver perchlorate gave the desired product after removal of the blocking groups from the initial product.

A number of trimethylsilyl derivatives of purines and pyrimidines were subsequently prepared by Nishimura and co-workers (118, 119) and


utilized for nucleoside synthesis (118-122). The pyrimidine derivatives
employed were all bis(trimethylsilyl) derivatives, the trimethylsilyl groups being attached to the oxygen at position 2 and the oxygen (for uracil and thymine derivatives) or amino group (for cytosine derivatives) at position 4. The purine derivatives employed were bis- and tris(trimethylsilyl) derivatives, one trimethylsilyl group being attached to the ring nitrogen at position 9 and the others being attached to oxygen or amino substituents on the purine ring. Examples of these derivatives are bis(trimethylsilyl)cytosine (XV) and 6-benzamido-bis-(trimethylsilyl)purine (XVI).

The procedure used by Nishimura and co-workers involved the fusion of a trimethylsilyl derivative of a purine or pyrimidine with an acylated glycosyl halide to give a blocked nucleoside, the trimethylsilyl group at O2 (of a pyrimidine) or at N-3 (of a purine) being replaced.
The remaining trimethylsilyl groups were easily removable by hydrolysis with water or alcohol. Wittenburg (123) introduced a slight modification in the procedure by carrying out the condensation in inert solvents (with and without suitable catalysts) as well as by fusion.

The nucleosides initially prepared by the trimethylsilyl procedure were D-ribofuranosyl (118, 121, 123), L-arabinofuranosyl (123), D-glucopyranosyl (118, 120, 122, 123) and D-galactopyranosyl (123) nucleosides. The yields obtained generally ranged from 40 to 55%. Subsequent applications of the trimethylsilyl procedure have been made for the synthesis of various additional nucleosides. The synthesis of pyrimidine nucleosides using this method has been particularly successful. In all cases where the sugar derivative contained a participating group at C-2, the C-1'-C-2'-trans anomers were obtained predominantly or exclusively.

5. Methods Involving Direct Condensation Between Purines or Pyrimidines and Sugar Derivatives

Included under this classification of synthetic methods are various procedures in which a sugar derivative is condensed directly with a free purine or pyrimidine derivative. In this case, a "free" purine or pyrimidine refers to one which does not contain a replaceable group (other than hydrogen) that is involved in the condensation reaction.

A procedure for direct condensation between per-O-acylglycosyl
halides and purine or pyrimidine bases has recently been reported by Yamaoka and co-workers (124). This procedure involves heating a mix-


ture of an acylated glycosyl halide and a purine or pyrimidine base in boiling nitromethane in the presence of mercuric cyanide as a hydrogen halide acceptor. D-Glucopyranosyl and D-ribofuranosyl nucleosides were the first to be synthesized using this procedure (124). The purines and pyrimidines employed were theophylline and other chloropurines, 6-benzamidopurine, and N6-benzoylcytosine. The yields of nucleosides obtained ranged from 43 to 94%.

Another method of this type that is finding increasing application involves the fusion of a sugar derivative (usually a fully acetylated sugar) with a purine in the presence of an acidic catalyst. This method is often referred to as the "fusion method".

The general procedure was first developed by Sato and co-workers (125, 126). They first fused D-ribose directly with theophylline and


other chloropurines in the presence of acidic catalysts and obtained nucleosides in low yield (125). It was found that improved yields
could be obtained by employing blocked sugars, such as methyl tri-O-acetyl-D-ribofuranoside or tetra-O-acetyl-D-ribofuranose, for the condensation reaction (126). These workers and others (127, 128)


found that, in general, the best yields could be obtained by employing fully acetylated sugars.

The fusion method was at first employed mainly for the synthesis of D-ribofuranosyl purine nucleosides (125-130) but was soon extended


to the synthesis of purine nucleosides containing various other sugar moieties such as D-arabino furanose (131), D-xylo furanose (132), D-


glucopyranose (131), D-galactopyranose (131), 2-deoxy sugars (133, 134),
and unsaturated sugars (25). A variety of purine derivatives have been employed for this method. Various catalysts have also been employed. These include polyphosphoric acid (135), p-toluene sulfonic acid (125–


127, 131–133, 135) and other sulfonic acids (128, 130), zinc chloride (130, 131, 135), sulfuric acid (127), sulfamic acid (129), and chloroacetic acid (134).

A method of nucleoside synthesis which requires only a free sugar and a purine or pyrimidine was developed by Schramm and co-workers (136, 137). This method consists of condensing the free sugar with a


free purine or pyrimidine base in N,N-dimethylformamide in the presence of ethyl polyphosphate. Using this method, these workers were able to synthesize adenosine (136), 2'-deoxyadenosine (136), 9-β-fructofuranosyladenosine (137), and 9-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-
adenine (137). The yields reported ranged from 15 to 30%. Subsequent attempts to further extend this method, however, met with some difficulty. For example, when Carbon (138) attempted to repeat the synthesis of 2'-deoxyadenosine using this method (136), he obtained six main products which absorbed in the ultraviolet region. Of these he obtained 2'-deoxyadenosine in only 6% yield and corresponding α-D-anomer in 25% yield.

The principal advantage in the methods of the type just described is that special derivatives of purines or pyrimidines (such as mercuri or trimethylsilyl derivatives) are not required. The methods are thus more direct and are especially useful in cases where a mercuri or other derivative of the desired aglycon is difficult to prepare. These direct procedures have often led to improved yields of nucleosides. A disadvantage of methods of this type, however, is that they are not generally applicable to all purine or pyrimidine derivatives. With these methods, as with all of the others discussed, the "trans rule" is followed (101, 102), that is, the C-1'-C-2'-trans-nucleoside is obtained predominantly or exclusively whenever a participating group is present at C-2 of the sugar derivative.

6. Stereospecificity in the Condensation Methods

In all of the condensation methods discussed, the "trans rule" (101, 102) was found to hold, that is, the entering nitrogen heterocycle will affix to the 1-position of the sugar exclusively or predominant-
ly on a side trans to the C-2 substituent, providing this substituent is a participating group (such as O-acyl). In most cases, these trans-nucleosides were the exclusive nucleosidic products.

There have been several explanations proposed for this phenomenon. Bristow and Lythgoe (139) proposed that the per-O-acylglycosyl halides


employed in all cases possessed a C-1-C-2-cis relationship and that the entering aglycon displaced the halogen atom with Walden inversion at C-1 thus forming the trans-nucleoside. It was subsequently shown, however, that many of the per-O-acylglycosyl halides employed possessed a C-1-C-2-trans relationship, especially in the cases of D-lyxo and D-arabino derivatives. It was also found (140) that both α- and β-D-


anomers of tetra-O-acetyl-D-glucopyranosyl chloride yielded the same nucleoside, 1-β-D-glucopyranosyluracil, when condensed with 2,4-dithiooxypyrimidine (after treatment of the initial product with methanolic hydrogen chloride). Thus the hypothesis of Bristow and Lythgoe (139) did not fully account for all of the results.

The possibility of a dual mechanism for C-1-C-2-cis and -trans sugar halides was raised by Howard (141). He proposed that, whereas

sugar halides with a C-1-C-2-cis-configuration react with simple Walden inversion, the C-1-C-2-trans sugar halides react by double Walden inversion, the C-1 configuration being initially inverted by participation of the C-2 acyloxy group to give an ortho-ester ion followed by a second inversion on reaction with the purine or pyrimidine derivative.

A more general explanation (which eliminates the necessity of proposing separate mechanisms for C-1-C-2-cis and -trans sugar halides) is that in the condensation reaction, C-1 of the sugar derivative assumes at least a partial carbonium ion character (the halide leaving before the attack of the aglycon).

The partial or full positive charge at C-1 is then delocalized by a participating group at C-2 favoring attack by the aglycon (B:) trans to the C-2 substituent.
Thus, a single mechanism could be operative regardless of the configuration of the glycosyl halide. This type of explanation is especially favorable for the Fischer-Helferich or mercuri procedures since the heavy metal salts could be expected to favor carbonium ion formation from the glycosyl halide by complexing with the halide. Further support for this type of mechanism comes from the fact that in several cases the condensation of a single anomeric sugar halide, which does not contain a participating group at C-2, with a purine or pyrimidine derivative was found to give both anomeric nucleosides. For example, the condensation of 3,4,6-tri-O-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl bromide with a 6-acetamido-9-chloromercuripurine gave 6-acetamido-9-[3,4,6-tri-O-acetyl-2-deoxy-2-(2,4-dinitroanilino)-β-D-glucopyranosyl] purine and the corresponding α-D anomer in a 5:3 ratio (31). Both anomers could be formed only if C-1 of the sugar derivative assumed a carbonium ion character, thus allowing attack of the purine derivative from either side (the 2,4-dinitroanilino group is a non-participating group). If the reaction were a simple Walden inversion only the formation of the β-D anomer would be expected.
7. Methods Involving Interconversions of Nucleosides

Methods of this type may be further classified into two general categories: (1) those involving alteration of the sugar moiety and (2) those involving alteration of the aglycon. The methods involving alteration of the sugar moiety generally employ procedures used for carbohydrates in general and usually involve inversion of an asymmetric carbon or replacement of a hydroxyl group with another substituent. These procedures will not be discussed here. A novel feature of some nucleoside interconversion methods, however, is the utilization of anhydronucleosides in the alteration of the sugar moiety. These anhydronucleosides are usually formed by the splitting off of an O-methanesulfonyl, O-tolylsulfonyl, or similar group from the sugar ring of a nucleoside and the formation of a bond between the sugar ring and the aglycon. For example, treatment of 1-(3,5-di-O-acetyl-2-O-tolylsulfonyl-β-D-ribofuranosyl)uracil (XVII) with methanolic ammonia gave the anhydronucleoside 2',5-anhydouridine (XVIII) (142).

Treatment of an anhydronucleoside with various nucleophilic reagents results in cleavage of the anhydro bridge between either the oxygen and aglycon (attack at the aglycon) or the oxygen and sugar (attack at the sugar ring) depending on the reagent and conditions employed. For example, treatment of 2',5-anhydouridine (XVIII) with dilute mineral acid gives 1-β-D-arabinofuranosyluracil (XIX) (attack at the aglycon) (142), while treatment of this same anhydronucleoside with sodium iodide in 2,5-hexanediol gives 1-(2-deoxy-2-iodo-β-D-ribofuranosyl)uracil (XX) (attack at the sugar ring) (143).

The principal application of methods of this type is for synthesis of C-1-C-2-cis nucleosides such as XIX above which are difficult to obtain by direct condensation methods, according to the "trans" rule (101, 102).

The second type of nucleoside interconversion method includes a variety of procedures used to alter the aglycon moiety. These procedures involve, for the most part, the replacement of hydrogen, carbonyl oxygen, or amino with various substituents. The most important of these procedures is the "thiation" procedure (53, 144) which involves the replacement of carbonyl oxygen of an aglycon with sulfur by treatment with phosphorus pentasulfide. The resulting thio derivative can then be
converted to a variety of derivatives by replacement of the sulfur with various groups.

8. Methods Involving Cyclization of N-Glycosyl Derivatives

A variety of procedures which involve the "construction" of the aglycon moieties of nucleosides are included under this classification of methods. Since methods of this type are not widely employed for nucleoside synthesis today, they will not be discussed in detail. All of these procedures have a common feature in that they begin with certain N-glycosyl derivatives (glycosylamines) which are cyclized to form nucleosides.

Methods of this type were first employed for the synthesis of purine nucleosides. The first of these methods was developed by Todd and co-workers (145-147). This method involved conversion of a 4,5-

(146) G. W. Kenner, B. Lythgoe, and A. R. Todd, ibid., 652 (1944).

- 6-(glycosylamino)pyrimidine (XXI) to the 5-thioformamide compound (XXII) followed by cyclization to the nucleoside (XXIII).
The nucleosides initially prepared by this method were 2-methyl-9-β-D-xylopyranosyladenine (144), 9-β-D-ribopyranosyladenine (145), 9-β-D-xylopyranosyladenine (146), and 2-methylthio-9-β-D-xylopyranosyladenine (147). Several other procedures of this type for the synthesis of purine nucleosides were subsequently developed. One of the methods, for example, involves cyclization of a 1-glycosylimidazole-4,5-dicarboxamide to the purine nucleoside by a modified Hofmann reaction with alkaline hypobromite (143).


The first cyclization method for the synthesis of pyrimidine nucleosides was developed by Shaw and co-workers (149, 150). This method consisted of reaction of a per-O-acylglycosylamine with a 2-cyano-3-ethoxy-η-(ethoxycarbonyl)acrylamide (XXIV) to give the intermediate XXV which then cyclizes to give a 5-cyanoacil nucleoside (XXVI).


This reaction was first applied to the synthesis of 5-cyanouracil nucleosides of D-glucose, D-galactose, D-xylose, and D-ribose (150). The development of a variety of other cyclization methods for the synthesis of pyrimidine nucleosides soon followed. Some of these include a method involving reaction of 3-ethoxyacryloyl isothiocyanate with a per-\(O\)-acylglycosylamine followed by treatment with sodium alkoxide to yield a 2-thiouracil nucleoside (151) and a method involving reaction of 5-cyano-1,3-thiazine with a \(per-O\)-acylglycosylamine to form a 5-cyano-2-thiouracil nucleoside (152).


Since these cyclization methods usually involve troublesome procedures and give low yields of nucleosides, they are not generally used for synthesizing nucleosides today, except in cases in which the desired nucleoside may be difficult to prepare by condensation methods. The main value of these methods is that they provided unambiguous evidence
of the position of the glycosyl linkage to the aglycon in naturally occurring nucleosides, since the synthesis of nucleosides was made by "construction" of the aglycon.

9. Nucleosides Synthesized by Various Methods

A large number and diversity of nucleosides have been synthesized. Only a limited listing of some representatives of the various types of nucleosides synthesized will be given here. A large number of pyrimidine nucleosides synthesized prior to 1959 have been tabulated by Fox and Wempen (53). Montgomery and Thomas (56) have made a similar tabulation of purine nucleosides synthesized prior to 1962.

Efforts to synthesize nucleosides were at first concentrated on the synthesis of naturally occurring nucleosides. More recent efforts have been concentrated on the synthesis of nucleosides containing interesting variations in the sugar or aglycon moiety. The mercuri method has been the most widely used of the synthetic methods.

All of the principal naturally occurring nucleic acid nucleosides have been synthesized by various methods. Cytidine was the first of these to be synthesized, the Hilbert-Johnson method being employed (109). Adenosine (103), guanosine (153), and 2'-deoxyguanosine (154)

(154) H. Venner, Ber., 22, 140 (1960).

were first synthesized using the Fischer-Helferich method. Uridine (155)
and 2'-deoxyctydine (156) were first synthesized by the mercuri method. Thymidine (156) and 2'-deoxyadenosine (157) were also first synthesized directly by the mercuri method but had been prepared earlier from other nucleosides by interconversion methods (158, 159).

Many other naturally occurring nucleosides have also been synthesized by various methods. For example, the nucleoside antibiotics puromycin (14) and nebularine (160) were synthesized by the mercuri method.


Pyrimidine (161) and purine (162) nucleosides of hexuronic acid
derivatives were first synthesized by the Hilbert-Johnson and Fischer-Helferich methods respectively.

The first nucleosides of ketosos to be synthesized were the adenine nucleosides of D-psicofuranose (24) and D-fructofuranose (and D-fructopyranose) (23). Both were synthesized using the mercuri method.

Nucleoside analogs containing the sugar moiety in the acyclic form have been synthesized in these laboratories using the mercuri method. The first of these to be synthesized were the 1-epimers of 1-(9-adenyl)-1-deoxy-1-O-methyl-D-galactose adhelydrol (163). The synthesis of nucleosides of disaccharidos was also first reported from these laboratories. In this case the mercuri method was employed for the synthesis of nucleosides of lactose (21), maltose (22), and cellobiose (22).

The synthesis of nucleosides of branched sugars has recently been reported (164). The mercuri procedure was employed to synthesize 9-
The synthesis of nucleosides of unsaturated sugars using various methods has been reported by several workers. For example, 7-(4,6-di-0-acetyl-2,3-dideoxy-D-erythro-hex-2-enopyranosyl)theophylline has been synthesized by the "fusion" method (25).

A number of nucleosides of amino sugars have been synthesized. The majority have been nucleosides of 3-amino-2-deoxy sugars. The synthesis of a nucleoside of a 3-amino-2-deoxy sugar, 9-(3-amino-3-deoxy-D-ribofuranosyl)-6-dimethylaminopurine, was first accomplished by Baker and co-workers (14) (using the mercuri method) as part of their total synthesis of puromycin. Examples of nucleosides of other types of amino sugars which have been synthesized are 1-(5-amino-5-deoxy-D-ribofuranosyl)thymine (165), 9-(3-amino-2,3-dideoxy-D-erythro-pentofuranosyl)-adenine (33), and 9-(6-amino-6-deoxy-D-glucopyranosyl)adenine (166).

The synthesis of nucleosides of 2-amino-2-deoxy sugars will be discussed in the following section.
III. Synthesis of Nucleosides of 2-Amino-2-deoxy Sugars

The synthesis of nucleosides of 2-amino-2-deoxy sugars has presented problems. The main difficulty has been the selection of an effective amino blocking group which can be conveniently introduced and easily removed. In addition, the blocking group should not interfere with the reactivity of the glycosyl halide in nucleoside formation.

A wide variety of amino blocking groups have been employed for amino sugars and amino acids. A list of some of the more important of these groups is given in table 1. Many of these groups have been employed only for amino acids (for peptide synthesis) and have not as yet been employed in the sugar series. Only some of the key references are given in table 1. For a more comprehensive list of references, as well as a more detailed discussion of these blocking groups, see Blosssonas (167) and Rudinger (168).


One of the main problems associated with the selection of an N-blocking group for 2-amino-2-deoxy sugars in nucleoside synthesis has been the difficulty in the removal of the N-blocking group from the blocked nucleoside after the condensation reaction, especially if the neighboring hydroxyl group is configurationally trans. In the synthesis of purine nucleosides of this type, the N-blocking groups employed must be removable under basic or neutral conditions since purine nucleosides are unstable to acid (52, 56). Thus, N-blocking groups such as alkoxy-
<table>
<thead>
<tr>
<th>Blocking Group</th>
<th>Methods of Removal</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzyloxycarbonyl and related groups:</td>
<td>Catalytic hydrogenolysis, sodium in liquid ammonia, HBr or HI in various solvents</td>
<td>169-177</td>
</tr>
<tr>
<td>[RCH₂CO₂]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R = phenyl, substituted phenoxy, or alkadienyl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkoxycarbonyl:</td>
<td>Treatment with various acids</td>
<td>172-179</td>
</tr>
<tr>
<td>[RO₂]</td>
<td></td>
<td>179-182</td>
</tr>
<tr>
<td>R = alkyl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3,5-Dimethoxybenzyloxycarbonyl:</td>
<td>Photochemical solvolysis</td>
<td>178</td>
</tr>
<tr>
<td>[3,5-(CH₃)₂C₆H₃CH₂O₂]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetyl and other acyl groups:</td>
<td>Vigorous acid hydrolysis, hot aqueous barium hydroxide</td>
<td>26-28</td>
</tr>
<tr>
<td>[RN]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetyl: R = CH₃</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formyl:</td>
<td>Vigorous acid hydrolysis, oxidation with H₂O₂</td>
<td>133, 134</td>
</tr>
<tr>
<td>[HO]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phthaloyl:</td>
<td>Hydrazine, stepwise alkaline and acid hydrolysis</td>
<td>185-187</td>
</tr>
<tr>
<td>Blocking Group</td>
<td>Methods of Removal</td>
<td>References</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>------------------------------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Trifluoroacetyl:</td>
<td>Basic hydrolysis or alcoholysis, acid hydrolysis</td>
<td>188-190</td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="Trifluoroacetyl" /></td>
<td></td>
</tr>
<tr>
<td>p-Tolylsulfonyl:</td>
<td>Sodium in liquid ammonia, acid hydrolysis</td>
<td>191, 192</td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="p-Tolylsulfonyl" /></td>
<td></td>
</tr>
<tr>
<td>Benzylsulfonyl:</td>
<td>Reduction with Raney Nickel, catalytic hydrogenolysis</td>
<td>193, 194</td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="Benzylsulfonyl" /></td>
<td></td>
</tr>
<tr>
<td>Tritylsulfenyl:</td>
<td>Acid hydrolysis</td>
<td>195</td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="Tritylsulfenyl" /></td>
<td></td>
</tr>
<tr>
<td>Thio-carbonyl derivatives:</td>
<td>Oxidation with H₂O₂ or peracids followed by acid hydrolysis, Treatment with basic lead salts</td>
<td>196</td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="Thio-carbonyl derivatives" /></td>
<td></td>
</tr>
<tr>
<td>2-p-Tolylsulfonyloethoxycarbonyl:</td>
<td>Basic hydrolysis or alcoholysis</td>
<td>197</td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="2-p-Tolylsulfonyloethoxycarbonyl" /></td>
<td></td>
</tr>
<tr>
<td>Modified Schiff Bases:</td>
<td>Vigorous acid hydrolysis</td>
<td>198, 199</td>
</tr>
<tr>
<td>5-chlorosalicylidene,</td>
<td><img src="image" alt="5-chlorosalicylidene" /></td>
<td></td>
</tr>
<tr>
<td>2-hydroxy-1-naphthal,</td>
<td><img src="image" alt="2-hydroxy-1-naphthal" /></td>
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</tr>
<tr>
<td>acetylisopropylidene,</td>
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</tr>
<tr>
<td>benzylisopropylidene,</td>
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<td>2-carboxyethylcyclopentylidene</td>
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<td></td>
</tr>
<tr>
<td>3-Keto-5,5-dimethylcyclohexylidene</td>
<td>Treatment with bromine water</td>
<td>200</td>
</tr>
<tr>
<td>Blocking Group</td>
<td>Methods of Removal</td>
<td>References</td>
</tr>
<tr>
<td>----------------</td>
<td>--------------------</td>
<td>------------</td>
</tr>
<tr>
<td>Trityl: (C₆H₅)₃C⁻</td>
<td>Catalytic hydrogenation, acid hydrolysis</td>
<td>201</td>
</tr>
<tr>
<td>2,4-Dinitrophenyl: 2,4-(NO₂)₂C₆H₃⁻</td>
<td>Basic (OH⁻) ion exchange resin, hot aqueous barium hydroxide</td>
<td>31, 35</td>
</tr>
<tr>
<td>Bis(phenoxyl)phosphinyl and bis(benzoxyl)phosphinyl: (RO)₂P⁻</td>
<td>Catalytic hydrogenolysis</td>
<td>202</td>
</tr>
</tbody>
</table>

R = phenyl or benzyl

(169) M. Bergmann and L. Zervas, Ber., 65, 1192 (1932).
(185) J. C. Sheehan and V. S. Frank, ibid., 71, 1856 (1949).
carbonyl or tritylsulfenyl could not be used for this type of synthesis. In the synthesis of pyrimidine nucleosides of 2-amino-2-deoxy sugars, the removal of the N-blocking group by catalytic hydrogenation could present problems since the pyrimidine aglycons themselves may be reduced (52, 53).

Another major source of difficulty associated with the selection of an amino blocking group is that a strongly participating N-blocking group at C-2 of the blocked glycosyl halide may interfere with its reactivity in the condensation reaction. Strongly participating groups at C-2 have been found to contribute to the instability of blocked 2-
amino-2-deoxy sugar halides (203). Such a group may displace the halide


from C-1 under the conditions of a condensation reaction to form an oxazolinium ion:

\[
\begin{array}{c}
\text{O} \\
\text{O} \\
\text{H} \\
\text{N} \\
\text{C} \quad \text{R} \\
\end{array}
\]

either by direct displacement of the halide by the N-blocking group or by initial formation of a carbonium ion at C-1 followed by participation of this group. However, unlike analogous ortho-ester ions, this intermediate could further react resulting in the decomposition of the sugar moiety. Thus, a 2-amino-2-deoxy-glycosyl halide containing a strongly participating N-blocking group might be unstable under the conditions of a condensation reaction resulting in a low or even zero yield of nucleoside. For example, attempted condensation of 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-a-D-glucopyranosyl bromide with the chloromercuri derivative of 6-dimethylamino-2-methylthiopurine did not yield any nucleoside because of the instability of the glycosyl halide under the reaction conditions (26). A glycosyl halide in which a strongly participating group at C-2 and the halide at C-1 bear a trans configurational relationship would be expected to be especially unstable, since the
geometry is especially favorable for a direct displacement of the halide by the group at C-2.

The N-acetyl group was the first amino blocking group to be employed in amino sugar nucleoside synthesis. Using this N-blocking group, Baker and co-workers (26) achieved the first synthesis of nucleosides of a 2-amino-2-deoxy sugar. By condensing 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranosyl chloride with 9-chloromercuri-6-dimethylamino-2-methylthiopurine, a blocked nucleoside derivative was obtained in 47% yield which was then de-O-acetylated to give 9-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-6-dimethylamino-2-methylthiopurine. This nucleoside was also reduced to give 9-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-6-dimethylaminopurine. Attempts to remove the N-acetyl group from these nucleoside derivatives were unsuccessful, however. Several other purine nucleosides of 2-amino-2-deoxy sugars were subsequently synthesized using acetyl as the amino blocking group (204, 205).


The N-acetyl group was not removed from any of these nucleoside derivatives, however.

Several years later, Baker and co-workers (28) were able to effect the removal of the N-acetyl blocking group with aqueous barium hydroxide to give a completely deblocked nucleoside of a 2-amino-2-deoxy sugar. In this work, 9-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-6-dimethylamino-
purine, which had previously been synthesized (26), was converted to 9-(2-acetamido-2-deoxy-\(\beta\)-D-allopyranosyl)-6-dimethylaminopurine by inversion of the C-3 hydroxyl group (by way of the 4,6-\(\Q\)-benzylidene-3-\(\Q\)-methanesulfonfonyl derivative). This nucleoside was then deblocked, in 36% yield, by refluxing aqueous barium hydroxide to give 9-(2-amino-2-deoxy-\(\beta\)-D-allopyranosyl)-6-dimethylaminopurine. A completely deblocked nucleoside of 2-amino-2-deoxy-D-ribose was also synthesized by this same general procedure (34). 2-Acetamido-3,5-di-\(\Q\)-acetyl-D-ribofuranosyl chloride was condensed with 9-chloromercuri-6-dimethylamino-2-methylthiopurine and the product reduced (to remove the 2-methylthio group of the purine), de-\(\Q\)-acetylated with methanolic ammonia or sodium methoxide in methanol, and de-\(\Q\)-acetylated with refluxing aqueous barium hydroxide to give 9-(2-amino-2-deoxy-\(\beta\)-D-ribofuranosyl)-6-dimethylaminopurine. This product was obtained in amorphous form of doubtful purity, however. Attempts to remove the \(\Q\)-acetyl group in nucleoside derivatives in which the acetamido group and neighboring hydroxyl group of the sugar moiety bore a trans configurational relationship were unsuccessful, however. The general procedure of Baker and co-workers was only successful for the synthesis of completely deblocked nucleosides of amino sugars in which the amino and neighboring hydroxyl groups bear a cis configurational relationship (14, 28, 34) and thus was not generally applicable.

The first synthesis of completely deblocked pyrimidine nucleosides of a 2-amino-2-deoxy sugar was achieved by Stevens and co-workers (27) who synthesized 1-(2-amino-2-deoxy-\(\beta\)-D-glucopyranosyl)cytosine and 1-(2-amino-2-deoxy-\(\beta\)-D-glucopyranosyl)uracil. In their work, acetyl was
initially employed as the amino blocking group. In this case it was possible to remove the \( \text{N}-\text{acetyl} \) group by vigorous acid hydrolysis, since pyrimidine nucleosides are stable to acid (52, 53). This procedure could not be used to remove \( \text{N}-\text{acetyl} \) from purine nucleosides, however, since they are unstable to acid (52, 56). These workers also employed the \( \text{N}-\text{methoxycarbonyl} \) and \( \text{N}-\text{benzyloxy carbonyl} \) blocking groups for the synthesis of these same nucleosides. The \( \text{N}-\text{methoxycarbonyl} \) group was removed by acid hydrolysis while the \( \text{N}-\text{benzyloxy carbonyl} \) group was removed by catalytic hydrogenation under mild conditions.

A method of removing the \( \text{N}-\text{acetyl} \) group in amino sugars under mild conditions has recently been reported (206). This method consists of

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\text{(206) S. Hanessian, Tetrahedron Letters, 1549 (1967).}
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treatment of the \( \text{N}-\text{acetyl} \) amino sugar derivative with triethylxonium fluoroborate at room temperature followed by treatment of the intermediate salt with dilute aqueous sodium bicarbonate to give the free amino sugar derivative. This method should be useful for removing the \( \text{N}-\text{acetyl} \) group in amino sugar nucleoside derivatives and could extend the usefulness of acetyl as an \( \text{N}-\text{blocking group} \) in amino sugar nucleoside synthesis. Another recently developed method of removing the \( \text{N}-\text{acetyl} \) group (207) involves treatment with phosphorus pentasulfide to give

\[
\text{(207) K. A. Watanabe, J. Beranek, H. A. Friedman, and J. J. Fox,}
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\[
\text{J. Org. Chem., 30, 2735 (1965).}
\]

\( \text{N}-\text{thioacetyl} \) which is then removed with methanolic ammonia at 100°.
This method cannot be used for nucleosides containing carbonyl groups in the aglycon which themselves could be converted to thiocarbonyl by the phosphorus pentasulfide unless this conversion is also desired.

A major disadvantage of the N-acetyl group is that it is strongly participating and is not generally applicable for use in the synthesis of nucleosides of all types of amino sugars. For example, 3,4,5,6-tetra-O-acetyl-2-acetamido-1-bromo-1,1,2-trideoxy-1-ethylthio-D-glucose aldehyde has been found to be too unstable to use in nucleoside synthesis (208).


Although the N-phthaloyl group has not been employed in synthesis of nucleosides of 2-amino-2-deoxy sugars, it is worthy of mention since it has been used as an amino blocking group in the synthesis of nucleosides of other types of amino sugars. This N-blocking was first employed by Baker and co-workers (209) in the synthesis of 9-(3-amino-3-


deoxy-β-D-ribofuranosyl)-6-dimethylamino-2-methylthiopurine. Condensation of 2,5-di-O-benzoyl-3-deoxy-3-phthalimido-D-ribofuranosyl chloride with 9-chloromercuri-6-dimethylamino-2-methylthiopurine followed by the de-O-benzoylation of the initial product gave 9-(3-deoxy-3-phthalimido-

β-D-ribofuranosyl)-6-dimethylamino-2-methylthiopurine. The N-phthaloyl
group was then removed by refluxing aqueous hydrazine to give 9-(3-amino-3-deoxy-β-D-ribofuranosyl)-6-dimethylamino-2-methylthiopurine. Treatment of this nucleoside with Raney Nickel gave the previously synthesized (14) 9-(3-amino-3-deoxy-β-D-ribofuranosyl)-6-dimethylamino-2-methylthiopurine.

Another amino blocking group employed for the synthesis of a nucleoside of a 2-amino-2-deoxy sugar is benzylsulfonyl (210). Condensation


of 3,4,6-tri-O-acetyl-2-N-(benzylsulfonyl)amino-2-deoxy-β-D-glucopyranosyl bromide with 6-acetamido-9-chloromercuripurine gave (after N-deacetylation of the initial product) 9-[3,4,6-tri-O-acetyl-2-N-(benzylsulfonyl)amino-2-deoxy-β-D-glucopyranosyl] adenine. However, attempted deblocking by treatment with Raney Nickel followed by O-deacetylation with methanolic ammonia gave only 9-(2-acetamido-2-deoxy-β-D-glucopyranosyl)adenine. Attempted deblocking by O-deacetylation followed by treatment with Raney Nickel was also unsuccessful since no nucleoside could be recovered after the final step. The N-benzylsulfonyl group thus appears to offer no advantage to N-acetyl as an amino blocking group for this type of synthesis.

The 2,4-dinitrophenyl group (a completely non-participating group) has been found to be extremely useful as an amino blocking group for synthesis of nucleosides of 2-amino-2-deoxy sugars. This group was first employed as an amino blocking group for amino sugars by Lloyd and co-workers (211). Several nucleosides of 2-amino-2-deoxy sugars have
been synthesized in these laboratories using 2,4-dinitrophenyl as the amino blocking group. The first application of this amino blocking group in amino sugar nucleoside synthesis was for the synthesis of the anomeric 9-(2-amino-2-deoxy-D-glucopyranosyl)adenines (31). Condensation of 3,4,6-tri-O-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl bromide with 6-acetamido-9-chloromercuripurine gave 6-acetamido-9-[3,4,6-tri-O-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl]-purine and the corresponding β-D anomeric in yields of 15 and 25%, respectively. After N-debenzoylation, the O-acetyl and N-2,4-dinitrophenyl groups were removed with strongly basic (OH⁻) ion exchange resin to give 9-(2-amino-2-deoxy-α-D-glucopyranosyl)adenine and the corresponding β-D anomeric in net yields of 1.7 and 2.4%, respectively, based on the blocked glucosyl bromide.

The N-2,4-dinitrophenyl blocking group was subsequently employed by Wolfson and Winkley in the synthesis of the anomeric adenine nucleosides of 2-amino-2-deoxy-D-glucofuranose (32) and of 2-amino-2-deoxy-D-ribofuranose (32, 35). 3,5,6-Tri-O-acetyl-2-deoxy-2-(2,4-dinitroanilino)-D-glucofuranosyl chloride (prepared from ethyl 2-deoxy-2-(2,4-dinitroanilino)-1-thio-α-D-glucofuranoside) was condensed with 6-acetamido-9-chloromercuripurine to give an anomeric mixture of blocked nucleoside derivatives which was deblocked with strongly basic (OH⁻) ion exchange resin. The resulting deblocked anomeric nucleosides were separated by elution from a column of ion-exchange
resin to give 9-(2-amino-2-deoxy-α-D-glucopyranosyl)adenine and the corresponding β-D anomer in net yields of 7 and 15%, respectively, based on the ethyl thio-glucopyranoside. Similarly, condensation of 3,5-di-O-acetyl-2-deoxy-2-(2,4-dinitroanilino)-D-ribofuranosyl chloride with 6-acetamido-9-chloromercaptpurine gave an anomeric mixture of blocked nucleoside derivatives which was deblocked and separated by elution from a column of ion-exchange resin to give 9-(2-amino-2-deoxy-α-D-ribofuranosyl)adenine and the corresponding β-D anomer in net yields of 20 and 19% respectively based on the blocked ribofuranosyl chloride.

The N-2,4-dinitrophenyl blocking group was also employed for the synthesis of a pyrimidine nucleoside of a 2-amino-2-deoxy sugar (114). In this work 3,4,6-tri-O-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl bromide was condensed with bis(trimethylsilyl)thymine to give 1-[tri-O-acetyl-2-deoxy-2-(2,4-dinitroanilino)-β-D-glucopyranosyl]-thymine. O-Deacetylation of this product with methanolic ammonia followed by removal of the N-2,4-dinitrophenyl group by treatment with refluxing concentrated aqueous barium hydroxide gave 1-(2-amino-2-deoxy-β-D-glucopyranosyl)thymine. Condensation of 3,4,6-tri-O-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl bromide with dithymylmercury, on the other hand, gave only a glycoside which could be converted into the blocked thymine nucleoside derivative in low yield by treatment with mercuric bromide in refluxing toluene.

The first synthesis of a nucleoside analog of an acyclic form of a 2-amino-2-deoxy sugar was accomplished using 2,4-dinitrophenyl as the amino blocking group (208). Condensation of 3,4,5,6-tetra-O-acetyl-1-
bromo-1,1,2-trIDEOxy-2-(2,4-dinitroanilino)-1-ethylthio-D-glucose aldehydrol was condensed with 6-acetamido-9-chloromercuripurine to give a blocked nucleoside derivative which was N-deacetylated to give 3,4,5,6-tetra-O-acetyl-1-(9-adenyl)-1,1,2-trIDEOxy-2-(2,4-dinitroanilino)-1-ethylthio-D-glucose aldehydrol. The corresponding 1-O-methyl and 1-O-ethyl derivatives were subsequently prepared in similar fashion and were then completely deacetylated (212). Attempts to remove the N-2,4-dini-


trophenyl group from these nucleoside derivatives were unsuccessful, however.

The principal advantage of the N-2,4-dinitrophenyl group is that it is completely non-participating. This not only contributes to the stability of the blocked glycosyl halides employed in nucleoside synthesis but also results in the formation of both anomeric nucleoside derivatives in most cases. Strongly basic conditions are required for the removal of this group, however.

The N-trifluoroacetyl group has recently been employed in these laboratories, with excellent results, as an amino blocking group in the synthesis of a completely deblocked nucleoside of a 2-amino-2-deoxy sugar (29, 30). Condensation of 3,4,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-β-D-glucopyranosyl bromide with bis(trimethylsilyl)-thymine gave 1-(3,4,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-β-D-glucopyranosyl)thymine in 80% yield. This product was completely de-blocked in one step with methanolic ammonia at room temperature to give, in 80% yield, 1-(2-amino-2-deoxy-β-D-glucopyranosyl)thymine.
Analogous experiments with the $N$-trichloroacetyl blocking group (29, 30) showed that this group required strong acid or hot aqueous barium hydroxide for removal and thus offered no advantage over the $N$-acetyl blocking group.

The results of this work indicate that trifluoroacetyl should be an excellent amino blocking group for amino sugar nucleoside synthesis. Its principal advantage is that it can be easily removed under mild conditions and in high yield by methanolic ammonia (which also removes O-acetyl blocking groups). Due to the electron withdrawing effects of the fluorine atoms, this group would not be expected to be strongly participating. The preliminary work (29, 30) with this group gave little indication of whether or not it shows any tendency to participate at C-1. The fact that only the $\beta$-D anomer was obtained in this case does not necessarily indicate that the $N$-trifluoroacetyl group participates at C-1 since the use of the non-participating $N$-2,4-dinitrophenyl group in the synthesis of this same nucleoside also resulted in the exclusive formation of the $\beta$-D anomer (114). It is possible that the use of the trifluoroacetyl group in the synthesis of other nucleosides of 2-amino-2-deoxy sugars may lead to the formation of anomeric nucleosides.
DISCUSSION OF RESULTS

I. Synthesis of Nucleosides of 2-Amino-2-deoxy-D-glucopyranose and 2-Amino-2-deoxy-D-galactopyranose

Due to recent interest in amino sugar nucleosides as possible antitumor and antibacterial agents, much effort has been directed toward the synthesis of this type of nucleoside. Efforts to synthesize nucleosides of 2-amino-2-deoxy hexoses have been concerned primarily with the synthesis of nucleosides of 2-amino-2-deoxy-D-glucose (26, 27, 29-32, 204, 205, 210), although a nucleoside of 2-amino-2-deoxy-allose, 9-(2-amino-2-deoxy-β-D-allopyranosyl)-6-dimethylaminopurine, has been synthesized (28).

Because of the limited work done in synthesizing nucleosides of 2-amino-2-deoxy hexoses other than 2-amino-2-deoxy-D-glucose, it was decided to undertake the synthesis of several nucleosides of 2-amino-2-deoxy-D-galactose. These nucleosides could then be investigated for possible anticancer properties.

Due to the high cost of 2-amino-2-deoxy-D-galactose, the synthesis of nucleosides of 2-amino-2-deoxy-D-glucose was first undertaken in order to develop procedures which could then be applied to the synthesis of nucleosides of 2-amino-2-deoxy-D-galactose. For this purpose, the synthesis of 1-(2-amino-2-deoxy-D-glucopyranosyl)cytosine and of 9-(2-amino-2-deoxy-D-glucopyranosyl)adenine was first undertaken.
For this work, it was initially decided to employ bis(phenoxyporphosphinyl) as the amino blocking group. N-Bis(alkoxy)phosphinyl, RNHPO(OR)₂, derivatives of amino acids and amino acid esters have been prepared for use in peptide synthesis (213). Bis(phenoxyporphosphinyl) has been employed as an amino blocking group in the sugar series for the synthesis of methyl 2-amino-2-deoxy-β-D-glucopyranoside (202). Removal of this group was accomplished by hydrogenation at high pressure in the presence of a platinum catalyst. This group was also converted to N-bis(benzyloxy)-phosphinyl (by ammonical benzyl alcohol) which could be removed by hydrogenation with a palladium catalyst at much lower pressure.

The synthesis of 1-(2-amino-2-deoxy-β-D-glucopyranosyl)cytosine was initially undertaken using the N-bis(phenoxyporphosphinyl) blocking group. This synthesis is illustrated in figure 10. The starting point for this synthesis was 3,4,6-tri-O-acetyl-2-N-[bis(phenoxyporphosphinyl)]-amino-2-deoxy-β-D-glucopyranosyl bromide (I) which was prepared by the procedure of Zervas and Kontzas (202). Initial attempts to prepare the desired nucleoside were made using the mercuri method. Condensation of I with N-acetylcytosinemercury (155) by the procedure of Fox and co-workers (155) gave N'-acetyl-1-[3,4,6-tri-O-acetyl-2-N-[bis(phenoxyporphosphinyl)]amino-2-deoxy-β-D-glucopyranosyl]cytosine (IIa). N'-Debenzoylation with picric acid in ethanol followed by depicration with
ion-exchange resin gave crystalline 1-[(3,4,6-tri-O-acetyl-2-N-[bis-(phenoxy)phosphinyl]amino-2-deoxy-β-D-glucopyranosyl)cytosine (IIb) in poor yield (8-9% based on the N-acetylcytosinemercury). Because of the poor yield obtained, this method was abandoned in favor of the trimethylsilyl method. Condensation of I with bis(trimethylsilyl)cytosine (119, 123) in refluxing benzene gave IIb in 75% yield based on I.

Attempts to remove the N-bis(phenoxy)phosphinyl group by catalytic hydrogenation, even at high pressures, were unsuccessful. Compound IIb was therefore treated with ammonical benzyl alcohol at room temperature to convert the N-bis(phenoxy)phosphinyl group to N-bis(benzyl oxy)-phosphinyl. This treatment also resulted in O-deacetylation to give, in 83% yield, crystalline 1-[(2-N-[bis(benzyl oxy)phosphinyl]amino-2-deoxy-β-D-glucopyranosyl)cytosine (IIc). Hydrogenation of IIc in the presence of palladium-charcoal catalyst with 92% aqueous dioxane or
90% aqueous methanol as solvent yielded crystalline 1-(2-amino-2-deoxy-β-D-glucopyranosyl)cytosine (III) in 33% yield. The hydrogenation of IIc to give III was accompanied by the formation of a second product, presumably one in which the cytosine moiety was reduced. Use of a more polar solvent system (higher percentage of water) led to increased production of this second product.

Concurrently with this work, Dr. E. J. Soltes, formerly of these laboratories, employed the N-bis(phenoxy)phosphinyl blocking group for the synthesis of a purine nucleoside, 9-(2-amino-2-deoxy-β-D-glucopyranosyl)adenine (214). Condensation of 3,4,6-tri-O-acetyl-2-N-[bis-


(phenoxyp)osphinyl]amino-2-deoxy-α-D-glucopyranosyl bromide (1) with 6-benzamido-9-chloromercuripurine gave 6-benzamido-9-[3,4,6-tri-O-acetyl-2-N-[bis(phenoxy)phosphinyl]amino-2-deoxy-β-D-glucopyranosyl]-purine. N-Debenzoylation with picric acid in ethanol followed by depicration with ion-exchange resin yielded 9-[3,4,6-tri-O-acetyl-2-

N-[bis(phenoxy)phosphinyl]amino-2-deoxy-β-D-glucopyranosyl]adenine. Treatment of this product with ammoniacal benzyl alcohol gave 9-[2-N-

[bis(benzyloxy)phosphinyl]amino-2-deoxy-β-D-glucopyranosyl]adenine which was deblocked by catalytic hydrogenation in the presence of palladium-charcoal catalyst and with 90% aqueous methanol as solvent to give 9-(2-amino-2-deoxy-β-D-glucopyranosyl)adenine in 93% yield from I.

The bis(phenoxy)phosphinyl group has thus proved to be a suitable N-protecting group in nucleoside synthesis with 2-amino-2-deoxy-D-
glucose. The direct use of the bis(benzylxyloxy)phosphinyl group throughout the entire synthetic procedure was precluded because a suitable reagent for the introduction of this group into the amino sugar was not available whereas bis(phenoxy)phosphinyl chloride, used to introduce the N-bis(phenoxy)phosphinyl group, was commercially available. It was thus necessary to initially employ the N-bis(phenoxy)phosphinyl group and to later convert this group into bis(benzylxyloxy)phosphinyl.

The overall yields of nucleosides obtained (based on I) were 20.5% for 1-(2-amino-2-deoxy-β-D-glucopyranosyl)cytosine and 9.3% for 9-(2-amino-2-deoxy-β-D-glucopyranosyl)adenine.

Because the yields of nucleosides obtained using the N-bis(phenoxy)-phosphinyl blocking group were relatively low and because of the problem of the formation of a second product during the hydrogenation of IIc to give 1-(2-amino-2-deoxy-β-D-glucopyranosyl)cytosine (presumably resulting from the reduction of the cytosine moiety), it was decided to investigate the use of trifluoroacetyl as the amino blocking group for this work. The synthesis of nucleosides of 2-amino-2-deoxy-D-glucopyranose and of 2-amino-2-deoxy-D-galactopyranose using trifluoroacetyl as N-blocking group is illustrated in figures 11-13. The trifluoroacetyl group has been employed in this laboratory, with excellent results, as an N-blocking group in the synthesis of 1-(2-amino-2-deoxy-β-D-glucopyranosyl)thymine (29, 30).

The synthesis of 1-(2-amino-2-deoxy-D-glucopyranosyl)cytosine and of 9-(2-amino-2-deoxy-D-glucopyranosyl)adenine was first undertaken. The key compound for this synthesis was 3,4,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-α-D-glucopyranosyl bromide (VIII) which could be
Fig. 11a. Synthesis of 3,4,6-Tri-O-acetyl-2-deoxy-2-trifluoroacetamido-α-D-glucopyranosyl Bromide and 3,4,6-Tri-O-acetyl-2-deoxy-2-trifluoroacetamido-α-D-galactopyranosyl Bromide
Fig. 12. Synthesis of Nucleosides of 2-Amino-2-deoxy-D-glucopyranose using Trifluoroacetyl as N-Blocking Group
Fig. 13. Synthesis of Nucleosides of 2-Amino-2-deoxy-D-galactopyranose
condensed with suitable purine or pyrimidine derivatives to give block-ed nucleosides. The glucosyl bromide (VIII) was first prepared by Hirschmann and co-workers (190) and employed for the synthesis of ethyl 2-amino-2-deoxy-β-D-glucopyranoside. These workers synthesized VIII by treatment of 3,4,6-tri-O-acetyl-2-amino-2-deoxy-β-D-glucopyranosyl bromide hydrobromide (ii) with trifluoroacetic anhydride. Compound ii, in turn, was prepared by treatment of 2-amino-2-deoxy-D-glucose (i) with acetyl bromide (215).


In this laboratory, 3,4,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-α-D-glucopyranosyl bromide was prepared by treatment of 1,3,4,6-tetra-O-acetyl-2-deoxy-2-trifluoroacetamido-β-D-glucopyranose (iv) with hydrogen bromide in acetic acid (29, 30). Compound iv, in turn, was prepared from 1,3,4,6-tetra-O-acetyl-2-amino-2-deoxy-β-D-glucose hydrochloride (iii), which was synthesized by the procedure of Bergmann and Zervas (216).
For the present work, a method of direct introduction of the \(H\)-trifluoroacetyl group into the amino sugar was desired. This would avoid the necessity of prior acetylation of the hydroxyl groups either by treatment with acetyl bromide, which is a troublesome procedure, or by methods similar to the one just described (involving acid hydrolysis of an acetylated Schiff base to yield the \(O\)-acetylated amino sugar salt) which requires five steps to synthesize the desired glycosyl halide from the amino sugar. A method of directly introducing the \(H\)-trifluoroacetyl group would be required for amino sugars for which no methods of prior selective \(O\)-acetylation are available.

In this work, a direct introduction of the \(H\)-trifluoroacetyl group was accomplished by selective \(H\)-trifluoroacetylation using \(S\)-ethyl trifluorothioacetate. This reagent has been employed for the introduction
of the \( \alpha \)-trifluoroacetyl group into amino acids for use in peptide synthesis (139). Treatment of a solution of 2-amino-2-deoxy-D-glucose in methanol gave 2-deoxy-2-trifluoroacetamido-D-glucose (Va). By repeated crystallization, the \( \beta \)-D anomor (Vb) was isolated. Acetylation of 2-deoxy-2-trifluoroacetamido-D-glucose (Va) with acetic anhydride-pyridine gave crystalline 1,3,4,6-tetra-O-acetyl-2-deoxy-2-trifluoroacetamido-\( \alpha \)-D-glucopyranose (VI) and the crystalline \( \beta \)-D anomor (VII) in yields of 40 and 45\%, respectively. Separation of the anomers was achieved by crystallization and preparative thin layer chromatography. Since VII was identical to the compound previously prepared in this laboratory (29, 30) from 1,3,4,6-tetra-O-acetyl-2-amino-2-deoxy-\( \beta \)-D-glucopyranose hydrochloride (iii), whose configuration had been unequivocally determined (217), the anomeric assignments of VI and VII could be made unequivocally. Compound VI was more dextrorotatory than VII thus showing a qualitative agreement with the Hudson rules of rotation (218). Treatment of either VI or VII with hydrogen bromide in acetic acid gave crystalline 3,4,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-\( \alpha \)-D-glucopyranosyl bromide (VIII). Compound VIII was also prepared from a mixture of VI and VII, thus avoiding the isolation of the individual anomeric acetates.
Condensation of 3,4,6-tri-0-acetyl-2-deoxy-2-trifluoroacetamido-
α-D-glucopyranosyl bromide (VIII) with bis(trimethylsilyl)cytosine
(119, 123) gave crystalline 1-(3,4,6-tri-0-acetyl-2-deoxy-2-trifluoro-
acetamido-β-D-glucopyranosyl)cytosine (XIV) in 42% yield by carrying
out the reaction in refluxing benzene or in 66% yield by fusion of the
two reactants. Complete deblocking of XIV was achieved in high yield
by treatment with methanolic ammonia for six days at room temperature
to give 1-(2-amino-2-deoxy-β-D-glucopyranosyl)cytosine (III). In all
work where only one anomer was obtained, the mother liquors were
investigated by thin layer chromatography for the other anomer.

Condensation of VIII with 6-benzamido-9-chloromercuripurine (109)
in refluxing toluene by the general procedure of Devoll and Lowy (109)
gave an anomeric mixture of blocked nucleoside derivatives which was
separable by preparative thin layer chromatography into crystalline
9-(3,4,6-tri-0-acetyl-2-deoxy-2-trifluoroacetamido-α-D-glucopyranosyl)-
purine (XVa) and the corresponding amorphous β-D anomer (XVIIa) in
yields of 7.3 and 26% respectively. Both XVa and XVIIa formed well
defined crystalline picrates (XVb and XVIIb) on η-debenzoylation with
picric acid in 2-propanol-methanol. Complete deblocking of XVa and
XVIIa was achieved in high yield by treatment with methanolic ammonia
for seven days at room temperature to give 9-(2-amino-2-deoxy-α-D-
glucopyranosyl)adenine (XVI) and the corresponding β-D anomer (XVIII).
Both XVI and XVIII had physical constants identifiable with the compounds
previously prepared in this laboratory (31). For each anomeric pair of
nucleoside derivatives (XVa-XVIIa, XVb-XVIIb, and XVI-XVIII) the a-D
anomer was the more dextrorotatory thus showing a qualitative agree­
ment with the Hudson rules of rotation (218).
The overall yields (based on VIII) of the nucleosides obtained using trifluoroacetyl as N-blocking group were 60% for 1-(2-amino-2-deoxy-β-D-glucopyranosyl)cytosine (III) and 6.5% and 22% for 9-(2-amino-2-deoxy-α-D-glucopyranosyl)adenine (XVI) and the corresponding β-D anomer (XVIII), respectively. Because of the excellent yields obtained and because of the convenience of the removal of the N-trifluoroacetyl group, it was decided to employ this blocking group for the synthesis of nucleosides of 2-amino-2-deoxy-D-galactose.

Treatment of a solution of 2-amino-2-deoxy-D-galactose in methanol produced 2-deoxy-2-trifluoroacetamido-D-galactose (Xa). By repeated crystallization, the α-D (Xb) anomer was isolated. Acetylation of 2-deoxy-2-trifluoroacetamido-D-galactose (Xa) with acetic anhydride-pyridine gave, after separation of the anomeric mixture by crystallization and preparative thin layer chromatography, crystalline 1,3,4,6-tetra-O-acetyl-2-deoxy-2-trifluoroacetamido-α-D-galactopyranose (XI) and the crystalline β-D anomer (XII) in yields of 46 and 37%, respectively. Since VI and VII gave a qualitative agreement with the Hudson rules of rotation (218) the anomeric assignments of XI and XII were made on the basis of optical rotation. Treatment of XI, XII, or a mixture of the two with hydrogen bromide in acetic acid gave 3,4,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-α-D-galactopyranosyl bromide (XIII).

3,4,6-Tri-O-acetyl-2-deoxy-2-trifluoroacetamido-α-D-galactopyranosyl bromide (XIII) was fused with bis(trimethylsilyl)cytosine to give, in 74% yield, amorphous 1-(3,4,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-β-D-galactopyranosyl)cytosine (XIX) which was initially isolated as the crystalline hydrate. Compound XIX was completely deblocked with meth-
anolic ammonia to give crystalline 1-(2-amino-2-deoxy-β-D-galactopyranosyl)cytosine (XX) in 91% yield.

The anomeric assignments of XIX and XX were made on the basis of the nmr spectrum of XX. This spectrum (measured in deuterium oxide) revealed a doublet at $\delta 5.58$ ($J = 9.4$ Hz) attributable to the anomeric proton ($H-1'$) of the sugar moiety. The magnitude of the coupling constant was indicative of a trans-diaxial relationship between $H-1'$ and $H-2'$ (219, 220). This relationship is found in the preferred conformation of the β-D anomer.


Fusion of 3,4,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-α-D-galactopyranosyl bromide (XIII) with bis(trimethylsilyl)thymine (119, 123) gave crystalline 1-(3,4,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-β-D-galactopyranosyl)thymine (XXI) in 80% yield. Deblocking of XXI with methanolic ammonia at room temperature gave amorphous 1-(2-amino-2-deoxy-β-D-galactopyranosyl)thymine (XXII) in 83% yield.

The anomeric assignments of XXI and XXII were made on the basis of
The nmr spectrum of XXII (measured in deuterium oxide) revealed a doublet at δ 5.52 (J = 9.4 Hz) attributable to the anomeric proton (H-1') of the sugar moiety. The magnitude of the coupling constant was indicative of a trans-diaxial relationship between H-1' and H-2' of the sugar moiety (219, 220) and thus of the β-D anomer.

Condensation of 3,4,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-α-D-galactopyranosyl bromide (XIII) with 6-benzamido-9-chloromeripurine in refluxing toluene by the general procedure of Davoll and Lowy (109) gave an anomic mixture of blocked nucleoside derivatives which was separable by preparative thin layer chromatography into crystalline 6-benzamido-9-(3,4,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-α-D-galactopyranosyl)purine (XXIIIa) and the corresponding amorphous β-D anomer (XXIVa) in yields of 9.3 and 30%, respectively. Both XXIIIa and XXIVa gave crystalline picrates (XXIIIb and XXIVb) on H-debenzoylation with picric acid in 2-propanol-methanol. Compounds XXIIIa and XXIVa were completely deblocked with methanolic ammonia to give 9-(2-amino-2-deoxy-α-D-galactopyranosyl)adenine (XXIIIc) and the corresponding β-D anomer (XXIVc) in yields of 83 and 81%, respectively. Both XXIIIc and XXIVc were obtained in amorphous form but gave crystalline dihydrochlorides (XXIIIId and XXIVd) on treatment with dilute hydrochloric acid in methanol. Since the adenine nucleoside derivatives of 2-amino-2-deoxy-D-glucopyranose (XVa-b, XVI, XVIIa-b, and XVIII) gave a qualitative
agreement with the Hudson rules of rotation (218), it was assumed that the corresponding derivatives of 2-amino-2-deoxy-D-galactopyranose (XXIIIa-d and XXIVa-d) would also give such an agreement. The anomic assignments of these compounds were thus made on the basis of optical rotation, XXIIIa-d being the more dextrorotatory of the anomic pairs.

The fact that anomic nucleoside derivatives were obtained on condensation of 6-benzamido-9-chloromercuripurine with VIII and with XIII is noteworthy since it indicates that the \( \text{N}-\text{trifluoroacetyl} \) group is not a strongly participating group. The \( \alpha: \beta \) anomic ratios were 0.25 for XVa: XVIIa and 0.33 for XXIIIa: XXIVa. A predominance of the \( \beta-D \) anomer would be expected in each case on steric grounds. The use of the non-participating \( \text{N}-2,4\text{-dinitrophenyl} \) group in a similar condensation reaction gave a much higher \( \alpha: \beta \) ratio, however. Thus, condensation of 6-acetamido-9-chloromercuripurine with 3,4,6-tri-\( O\)-acetyl-2-deoxy-2-(2,4-dinitroanilino)-\( D \)-glucopyranosyl bromide gave an anomic mixture of blocked nucleoside derivatives in an \( \alpha: \beta \) ratio of 0.6 (31). This indicates that the \( \text{N}-\text{tri-fluoroacetyl} \) group may participate to a small extent at C-1, giving an increased proportion of the \( \beta-D \) anomer. No firm conclusions can be drawn in this respect from this limited evidence, however.

The fact that condensation of the blocked glycosyl halides VIII and XIII with bis(trimethylsilyl)cytosine and with bis(trimethylsilyl)-thymine, in contrast to the condensation with 6-benzamido-9-chloromercuripurine, yielded only the \( \beta-D \) anomers may be due to a difference in reaction mechanisms. Whereas in the case of the condensation with 6-benzamido-9-chloromercuripurine, C-1 of the sugar probably assumes a
partial or complete carbonium ion character allowing attack by the aglycon from both sides and thus giving both anomers, the condensation with the trimethylsilyl derivatives may be more of a direct displacement of the halide with Walden inversion. Thus, since the α-D glycosyl bromides were employed, a direct displacement with Walden inversion would give the β-D anomic nucleoside derivatives. This conclusion is supported by the fact that condensation of 3,4,6-tri-O-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl bromide with bis(trimethylsilyl)thymine yielded only the β-D anomic nucleoside derivative (114).

II. Synthesis of 2-Amino-1,1,2-trideoxy-1-ethythio-1-(1-thyminyl)-D-glucose Aldehydrol

With few exceptions, the acyclic aldehyde (v) and aldehydrol (vi) forms of sugars are so unstable that they exist only in aqueous solution.

A sugar may be forced into the aldehyde form by blocking of the hydroxyl groups. Stable acyclic sugar derivatives may also be obtained in which halogens, oxyacyl, oxyalkyl, and thio-oxyalkyl groups are substituted for the hydroxyl groups of the aldehydrol form (vi). Several acyclic sugar nucleoside analogs have been prepared in these laboratories which may be considered to be derived from the aldehydrol forms of various sugars (163, 208, 212, 221, 222). These derivatives may
also be considered as nucleoside derivative of acyclic sugar hemiacetals (163, 212, 222) or acyclic sugar thiohemiacetals (208, 221).

These acyclic sugar nucleoside analogs are of value not only from the standpoint of being interesting compounds in themselves but also because it may be possible to cleave the alkoxy or thioalkoxy group at C-1 of the sugar moiety resulting in cyclization of the sugar to the furanose or pyranose form. Several attempts to cyclize acyclic sugar analogs by this procedure have been made in these laboratories but have all been unsuccessful. For example, attempts to cyclize 1-(9-adenyl)-1-0-benzyl-1-deoxy-D-galactose aldehydrol by cleavage of the O-benzyl group with sodium in liquid ammonia or by catalytic hydrogenation were unsuccessful (222).

Only three such nucleoside analogs of an amino sugar have been synthesized: 1-(9-adenyl)-1,2-dideoxy-2-(2,4-dinitroanilino)-1-0-ethyl-D-glucose aldehydrol (212) and the corresponding 1-0-methyl (212) and 1-ethythio (208) derivatives. The N-2,4-dinitrophenyl blocking group could not be removed from these derivatives, however. It was therefore decided to attempt the synthesis of a completely deblocked nucleoside analog of an acyclic form of 2-amino-2-deoxy-D-glucose using trifluoroacetetyl as the amino blocking group. This synthesis is illustrated in figure 14.
Fig. 14. Synthesis of 2-Amino-1,1,2-trideoxy-1-ethythio-1-(1-thymynyl)-D-glucose Aldehydrol
The first consideration was the synthesis of a suitably blocked acyclic halide of 2-amino-2-deoxy-D-glucose which could be condensed with a purine or pyrimidine derivative. For this purpose, it was decided to employ the Gauthier bromination procedure (223) which consists of the treatment of a suitably blocked diethyl dithioacetal with a molar equivalent of bromine.

\[
\begin{align*}
\text{HC(SEt)}_2 & \quad \text{Br}_2 & \quad \text{BrCH(SET)}_2 \\
(\text{CHOR})_4 & \quad \text{CH}_2\text{OR} & \quad (\text{CHOR})_4 \\
\end{align*}
\]

The starting point of this synthetic procedure was 2-amino-2-deoxy-D-glucose diethyl dithioacetal hydrochloride (XXV) which was prepared by the procedure of Kent (224) as modified by Hough and Taha (225). A methanolic solution of the free amino form of this compound (formed by treatment of the hydrochloride with a molar equivalent of sodium methoxide) was treated with 2-ethyl trifluoroacetate to give crystalline 2-deoxy-2-trifluoroacetamido-D-glucose diethyl dithioacetal (XXVI) in 73% yield. Compound XXVI was acetylated with acetic anhydride-pyridine
to give, in 92% yield, 3,4,5,6-tetra-O-acetyl-2-deoxy-2-trifluoroacetamido-D-glucose diethyl dithioacetal (XXVII).

Treatment of a solution of XXV in ether with slightly more than one molar equivalent of bromide gave tetra-O-acetyl-1-bromo-1,1,2-trideoxy-1-ethylthio-2-trifluoroacetamido-D-glucose aldehydrol (XXVIII) as a syrup. Crystallization of the crude bromide was not attempted but the crude product was immediately condensed with bis(trimethylsilyl)thymine by fusion to give a mixture of the 1'-epimeric nucleoside derivatives. Separation by preparative thin layer chromatography yielded amorphous 3,4,5,6-tetra-O-acetyl-1,1,2-trideoxy-1-ethylthio-1-(thyminy1)-D-glucose aldehydrol, (+) form, (XXIX) and the corresponding crystalline epimeric (-) form (XXXI) in 15 and 26% yield respectively based on XXVII. These two forms are designated (+) and (-) according to the sign of their optical rotations.

Deacylation of XXIX with methanolic ammonia at room temperature gave 2-amino-1,1,2-trideoxy-1-ethylthio-1-(1-thyminy1)-D-glucose aldehydrol, (+) form, (XXXa) as a white amorphous solid which quickly took up moisture from the air and became a gum. Since this compound was difficult to handle, it was not characterized but was immediately converted to the stable amorphous hydrochloride (XXXb) by dilute hydrochloric acid in methanol. Similarly, deblocking of XXXI with methanolic ammonia gave amorphous 2-amino-1,1,2-trideoxy-1-ethylthio-1-(1-thyminy1)-D-glucose aldehydrol, (-) form (XXXIIa) which was also immediately converted to the amorphous hydrochloride (XXXIIb).

The acyclic sugar nucleoside analogs prepared in the present work could possibly be cyclized by removal of the ethylthio group at C-1
of the sugar moiety provided suitable procedures could be developed for this purpose. Attempts to demercaptalate these compounds were not made in the present work. However, attempts to cyclize other acyclic sugar nucleoside derivatives containing ethylthio groups at C-1 of the sugar moiety by demercaptalation were made in these laboratories. All of these attempts were unsuccessful.

III. Synthesis of 1-(2-Amino-2-deoxy-\(\beta\)-D-glucofuranosyl)cytosine

Since the sugar moieties of the naturally occurring nucleosides are of the furanose form, it was considered desirable to synthesize nucleosides of the furanose form of 2-amino-2-deoxy sugars. Thus far, the only nucleosides of the furanose form of a 2-amino-2-deoxyhexose to be synthesized are the anomeric 9-(2-amino-2-deoxy-D-glucofuranosyl)-adenines (32). It was thus decided to undertake the synthesis of a pyrimidine nucleoside of the furanose form of 2-amino-2-deoxy-D-glucose. This synthesis is illustrated in figure 15.

The key compound for this synthesis was a suitably blocked halide of 2-amino-2-deoxy-D-glucofuranose which could be condensed with a suitable pyrimidine derivative. A pentose may be forced into its furanose form by blocking of the 5-hydroxyl group. This type of procedure is not generally applicable to the hexose series, however. Another method for forcing a sugar into its furanose form, which is applicable to the hexose series, is the partial demercaptalation of a sugar diethyl dithioacetal. This type of method was employed by Pacsu and Wilson (226).
Fig. 15 Synthesis of 1-(2-Amino-2-deoxy-β-D-glucofuranosyl)cytosine
to synthesize ethyl 1-thio-α-D-glucofuranoside by partial demercaptalysis of D-glucose diethyl dithioacetal with aqueous mercuric chloride in the presence of mercuric oxide. This general procedure was applied to a 2-amino-2-deoxy sugar by Wolfrom, Olin, and Polglase (227). By partial demercaptalysis of 2-acetamido-2-deoxy-D-glucose diethyl dithioacetal, they obtained ethyl 2-acetamido-2-deoxy-1-thio-α-D-glucofuranoside and the corresponding β-D anomer (as its triacetate). This procedure has recently been modified by Wolfrom and Winkley (228).

Wolf from and Winkley employed ethyl 2-acetamido-2-deoxy-1-thio-α-D-glucofuranoside (as its triacetate) as the starting point for the synthesis of the anomic 9-(2-amino-2-deoxy-D-glucofuranosyl)adenines (32). This compound was deacetylated and the N-2,4-dinitrophenyl group introduced. Acetylation of the resulting compound gave ethyl 3,5,6-tri-O-acetyl-2-deoxy-2-(2,4-dinitroanilino)-1-thio-α-D-glucofuranoside which was converted into the glycosyl chloride by displacement of the 1-
ethylthio group with chlorine. Condensation of this glycosyl chloride with 6-acetamido-9-chloromercuripurine followed by deblocking gave the desired nucleosides.

For the present work, trifluoroacetyl was again chosen as the amino blocking group. It was decided to employ the same type of general procedure as used by Wolf and Winkley (32, 228) in which a suitably blocked 2-amino-2-deoxy-D-glucose diethyl dithioacetal is partially demercaptalated to obtain ethyl 2-amino-2-deoxy-1-thio-D-glucofuranoside (in suitably blocked form) which is then treated with chlorine to give the desired glycosyl halide. Two alternate routes to the N-blocked ethyl 2-amino-2-deoxy-1-thio-D-glucofuranoside were investigated. The first involves introduction of the N-trifluoroacetyl group into the diethyl dithioacetal before demercaptalation to give the desired thio-glucofuranoside directly upon demercaptalation. The second involves the introduction of the N-trifluoroacetyl group after the demercaptalation step, ethyl 2-acetamido-2-deoxy-1-thio-α-D-glucofuranoside being first prepared and deacetylated, then the N-trifluoroacetyl group introduced.

Partial demercaptalation of 2-deoxy-2-trifluoroacetamido-D-glucose diethyl dithioacetal (XXVI) with aqueous mercuric chloride in the presence of mercuric oxide by the general method of Pascu and Wilson (226) produced crystalline ethyl 2-deoxy-1-thio-2-trifluoroacetamido-α-D-glucofuranoside (XXXIII) in 37% yield. This compound was also prepared in 70% yield by deacetylation of ethyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-1-thio-α-D-glucofuranoside (XXXIV) followed by treatment of the resulting ethyl 2-amino-2-deoxy-1-thio-α-D-glucofuranoside (XXXV) with S-ethyl trifluorothioacetate. The deacetylation of XXXIV was effected
by refluxing 15% aqueous barium hydroxide. The 2-acetamido-3,4,6-tri-
\(\text{\text{-}\text{\text{-}\text{-}\text{\text{-}\text{-}\text{\text{-}\text{-}\text{\text{-}\text{-}\text{\text{-}}}3,6-tri-}
\text{\text{-}\text{\text{-}\text{\text{-}\text{-}\text{\text{-}\text{-}\text{\text{-}}}2-deoxy-1-thio-\alpha-D-glucofuranoside}
employed for this purpose was prepared by partial demercaptalation of
2-acetamido-2-deoxy-\text{\text{-}\text{\text{-}\text{-}\text{-}\text{-}\text{\text{-}\text{-}\text{-}\text{-}}}glucose diethyl dithioacetal by the general procedure of Wolfrom,
Olin, and Polglase (227) as modified by Wolfrom and Winkley (228).
Ethyl 2-deoxy-1-thio-2-trifluoroacetamido-\alpha-D-glucofuranoside (XXXIII)
was acetylated with acetic anhydride-pyridine to give crystalline
ethyl 3,4,6-tri-\text{\text{-}\text{\text{-}\text{\text{-}\text{-}\text{-}\text{-}\text{-}\text{-}\text{-}\text{-}}}2-deoxy-1-thio-2-trifluoroacetamido-\alpha-D-
glucofuranoside (XXXVI) in 91% yield.

Treatment of a solution of XXXVI in dichloromethane with dry
chlorine gave syrupy 3,4,6-tri-\text{\text{-}\text{\text{-}\text{-}\text{-}\text{-}\text{-}\text{-}\text{-}\text{-}\text{-}3,4,6-tri-}
\text{\text{-}\text{\text{-}\text{-}\text{-}\text{-}\text{-}\text{-}\text{-}\text{-}}}2-deoxy-2-trifluoroacetamido-D-
glucofuranosyl chloride (XXXVII) which was immediately condensed with
bis(trimethylsilyl)cytosine by fusion. Crystallization of the crude
product from this reaction gave 1-(3,4,6-tri-\text{\text{-}\text{\text{-}\text{-}\text{-}\text{-}\text{-}\text{-}\text{-}\text{-}\text{-}}}3,4,6-tri-
fluoroacetamido-\beta-D-glucofuranosyl)cytosine (XXXVIII) in 12% yield. An
additional 2.3% of crystalline XXXVIII was isolated from the mother
liquors by preparative thin layer chromatography. Attempted isolation
of a second anomeric nucleoside derivative from these mother liquors
by preparative thin layer chromatography was unsuccessful. The major
components instead proved to be non-nucleosidic material. The blocked
nucleoside (XXXVIII) was deacylated with methanolic ammonia at room
temperature to give amorphous 1-(2-amino-2-deoxy-\beta-D-glucofuranosyl-
cytosine (XXXIXa) which was immediately converted into the crystalline
sulfate (XXXIXb) in 85% yield from XXXVIII.

The anomeric assignments of XXXVIII, XXXIXa and XXXIXb were made
on the basis of optical rotatory dispersion. It has recently been
found (229, 230) that the optical rotatory dispersion spectra of β-D


pyrimidine nucleosides of the furanose form exhibit positive Cotton effects while the spectra of the corresponding α-D anomers exhibit negative Cotton effects. The optical rotatory dispersion spectrum of XXXIXb (see figure 16) was found to exhibit a positive Cotton effect, with $\lambda_{\text{max}}$ at 282 nm ([M] +9,000°) and $\lambda_{\text{min}}$ at 238 nm ([M] -13,500°), and was thus concluded to be the β-D anomer.

Because of the low yield of nucleoside obtained, an attempt was made to prepare XXXIX, in possible higher yield, by employing the completely non-participating N-2,4-dinitrophenyl blocking group. Ethyl 3,5,6-tri-O-acetyl-2-deoxy-2-(2,4-dinitroanilino)-1-thio-α-D-glucofuranoside (32) was converted into the glycosyl chloride (32) by treatment with dry chlorine and this was immediately fused with bis-(trimethylsilyl)cytosine. A nucleoside was not isolated from this reaction, however. Separation of the crude product by preparative thin layer chromatography revealed that any nucleoside derivative, if present, was present in small quantity.
Fig. 16. Optical Rotatory Dispersion Spectrum of 1-(2-Amino-2-deoxy-β-D-glucofuranosyl)cytosine Sulfate

IV. Synthesis of 1-(2-Amino-2-deoxy-β-D-xylofuranosyl)thymine

Thus far only three nucleosides of 2-amino-2-deoxypentoses have been synthesized. All of these are nucleosides of 2-amino-2-deoxy-D-ribose: the anomeric 9-(2-amino-2-deoxy-D-ribofuranosyl)adenines (32, 35) and 9-(2-amino-2-deoxy-β-D-ribofuranosyl)-6-dimethylaminopurine (34). In addition, a nucleoside of a 2-amino-2,3-dideoxypentose, 9-(2-amino-2,3-dideoxy-β-D-erythro-pentofuranosyl)adenine, has been synthesized by
a nucleoside interconversion method (33). No pyrimidine nucleoside of
a 2-amino-2-deoxypentose has as yet been synthesized. It was thus
decided to undertake the synthesis of a pyrimidine nucleoside of a 2-
amino-2-deoxy-pentose of the furanose form. Trifluoroacetyl was again
chosen as the amino blocking group for this synthesis.

Since the 2-amino-2-deoxypentoses are not readily available, the
first problem was to synthesize a suitable quantity of a 2-amino-2-
deoxy-pentose, or a derivative thereof, to be employed for this syn-
thesis. The second problem was to force the pentose into its furanose
form. One method for synthesizing a 2-amino-2-deoxypentose is to
cleave the C-5-C-6 bond of a 2-amino-2-deoxyhexose. This type of
method was employed by Wolf from and Anno (231) for the synthesis of

(1953).

ethyl 2-acetamido-2-deoxy-1-thio-α-D-xylofuranoside from ethyl 2-
acetamido-2-deoxy-1-thio-α-D-glucofuranoside by oxidative cleavage
between C-5 and C-6 followed by reduction of the product.

Since this method gives a 2-amino-2-deoxy-D-xylose derivative of the
furanose form it was decided to employ the general procedure of Wolf from
and Anno (231) for the synthesis of a 2-amino-2-deoxy-D-xylose derivative and to use this derivative as the starting point in the synthesis of a pyrimidine nucleoside of 2-amino-2-deoxy-D-xylofuranose. This synthesis is represented in figure 17.

![Chemical structures](image)

Fig. 17. Synthesis of 1-(2-Amino-2-deoxy-β-D-xylofuranosyl)thymine

The problem was greatly simplified by the fact that a suitable derivative of 2-amino-2-deoxy-D-glucose of the furanose form (ethyl 2-deoxy-1-thio-2-trifluoroacetamido-α-D-glucofuranoside, XXXIII), which already contains the N-trifluoroacetyl blocking group, had been synthesized in the sequence described in the preceding section. The 5-6 bond of this compound could thus be cleaved to yield a 2-amino-2-deoxy-D-xylose
derivative of the furanose form, which was the starting point for this synthesis. Following the general procedure of Wolfrom and Anno (231) as modified by Wolfrom and Winkley (228), ethyl 2-deoxy-1-thio-2-trifluoroacetamido-α-D-glucofuranoside (XXXIII) was treated with slightly more than one molar equivalent of sodium metaperiodate and the product immediately reduced with sodium borohydride to give crystalline ethyl 2-deoxy-1-thio-2-trifluoroacetamido-α-D-xylofuranoside (XL) in 73% yield. Acetylation of XL with acetic anhydride-pyridine gave crystalline ethyl 3,5-di-O-acetyl-2-deoxy-1-thio-2-trifluoroacetamido-α-D-xylofuranoside (XLI). Treatment of XLI in dichloromethane with dry chlorine gave syrupy 3,5-di-O-acetyl-2-deoxy-2-trifluoroacetamido-D-xylofuranosyl chloride (XLII) which was immediately fused with bis-(trimethylsilyl)thymine (119, 123). The product of this reaction was purified by preparative thin layer chromatography to give amorphous 1-(3,5-di-O-acetyl-2-deoxy-2-trifluoroacetamido-β-D-xylofuranosyl)-thymine (XLIII) in 61% yield. Compound XLIII was deacylated with methanolic ammonia to give amorphous 1-(2-amino-2-deoxy-β-D-xylofuranosyl)thymine (XLIVa) which was immediately converted to the crystalline hydrochloride (XLIVb) in 86% yield from XLIII.

The anomeric assignments of XLIII, XLIVa, and XLIVb were made on the basis of optical rotatory dispersion. The optical rotatory dispersion spectrum of XLIVb in water (see figure 18) exhibited a positive Cotton effect with max at 283 nm ([M] +4,400°) and min at 252 nm ([M] -8,000°). This positive Cotton effect is characteristic (229, 230) of the β-D configuration in pyrimidine nucleosides of the furanose form.
Fig. 18. Optical Rotatory Dispersion Spectrum of 1-(2-Amino-2-deoxy-β-D-xylofuranosyl)thymine Hydrochloride
Experimental (232)

(232) Melting points were determined with a Thomas-Hoover apparatus. Specific rotations were determined in a 2-dm polarimeter tube. Infrared spectra were measured with a Perkin-Elmer Infra cord spectrometer. Ultraviolet spectra were measured with a Bausch and Lomb Spectronic 505 spectrometer. Optical rotatory dispersion spectra were measured with a Jasco ORD/UV5 spectrometer. Nmr spectra were recorded by Dr. T. Radford with a Varian A-60 nmr spectrometer. X-ray powder diffraction data give interplanar spacings in angstroms for CuKα radiation. Relative intensities were estimated visually: s, strong; m, moderate; w, weak; v, very. The stronger lines are numbered (1, strongest); multiple numbers indicate approximately equal intensities. Thin layer chromatography was performed with Desaga equipment using silica gel G (E. Merck, Darmstadt, Germany) activated at 110°. Indication was by sulfuric acid unless otherwise noted; amounts of developers given are by volume. Microanalyses were performed by W. N. Rond. Unless otherwise indicated, evaporations were performed under diminished pressure (water aspirator).
I. Synthesis of Nucleosides of 2-Amino-2-deoxy-D-
glucopyranose and 2-Amino-2-deoxy-D-galactopyranose

1. 1-[3,4,6-Tri-O-acetyl-2-N-[bis(phenoxy)phosphinyl]amino-
2-deoxy-β-D-glucopyranosyl]cytosine (IIb)

(A) Attempts to prepare N4-acetyl-1-[3,4,6-tri-O-acetyl-2-deoxy-
2-N-[bis(phenoxy)phosphinyl]amino-β-D-glucopyranosyl]cytosine (IIa) by condensing I with N-acetylcytosinemercury in either refluxing benzene or toluene were made using different reflux times (1-5 hr). The glycosyl bromide was added in two equal portions, the second after 0.5 to 1 hr. The yields in all trials were similar. A typical condensation follows.

A mixture of N-acetylcytosinemercury (0.44 g, 0.00125 mole) and toluene (30 ml) was azeotropically dried by distillation of approximately ½ of the solvent. 3,4,6-Tri-O-acetyl-2-deoxy-2-N-[bis(phenoxy)-phosphinyl]amino-α-D-glucopyranosyl bromide (202) (I, 0.75 g, 0.00125 mole) was added, with stirring, and the mixture was refluxed for 1 hr. An additional 0.75 g (0.00125 mole) of the bromide (I) was added and the mixture was refluxed for an additional 2 hr. The reaction mixture was cooled and poured into petroleum ether (bp 30-60°, 100 ml). The precipitate which formed was filtered and extracted with chloroform. The chloroform solution was washed with 30% aqueous potassium iodide and water, then dried over anhydrous sodium sulfate. Evaporation of the solvent gave a solid residue (0.90 g) which was dissolved in ethanol (20 ml) and picric acid (0.3 g) added. The solution was refluxed for 30 min then refrigerated overnight. A yellow solid formed which was filtered and washed with ether. This picrate was dissolved in 90%
aqueous acetone (40 ml) and stirred with a slight excess of Dowex AG 1-X2 (CO₃⁻²) resin. The resin was removed by filtration and washed with acetone (50 ml). The combined filtrate and washings were evaporated to dryness and the residue was crystallized twice from ethanol-ether, yield 60 mg (8% based on the H-acetylcystosinemercury): mp 260°C dec with softening above 150°C; [α]²⁰⁺₁₂ ± 1° (c 1.5, methanol).

(B) 3,4,6-Tri-O-acetyl-2-N-[bis(phenoxy)phosphinyl]amino-2-deoxy-a-D-glucopyranosyl bromide (I, 3.0 g) was added over a period of 15 min to a stirred and gently heated mixture of bis(trimethylsilyl)cytosine (119, 123) in dry benzene (130 ml). The mixture was then refluxed, with stirring, for 2 hr. The white precipitate which formed was filtered and added to 70% aqueous ethanol (50 ml) containing sodium bicarbonate (1.0 g). After heating for 15 min at 60°C the solvent was evaporated and the residue extracted with chloroform. Evaporation of the chloroform extract to dryness yielded a white residue which was crystallized from methanol-ether, yield 2.37 g (75%): mp 260°C dec with softening at 155-170°C, [α]²²⁺₁₂ ± 1° (c 3.2, methanol).

Anal. Calcd for C₂₈H₃₁H₇O₇N₄P: C, 53.33; H, 4.96; N, 8.89. Found: C, 53.46; H, 4.97; N, 8.91.

2. 1-{2-N-[Bis(benzyloxy)phosphinyl]amino-2-deoxy-β-D-glucopyranosyl}cytosine (IIc)

1-{3,4,6-Tri-O-acetyl-2-N-[bis(phenoxy)phosphinyl]amino-2-deoxy-β-D-glucopyranosyl}cytosine (IIb, 2.7 g) was dissolved in 50 ml of benzyl alcohol nearly saturated with ammonia at 0°C. The solution was kept for 48 hr at room temperature, after which it was heated for 15 min at 60°C under water aspirator vacuum to remove excess ammonia. The
precipitate obtained on pouring the solution into 500 ml of ether was dissolved in 20 ml of methanol and reprecipitated in ether. Crystallization from 95% methanol gave a white crystalline material, yield 1.9 g (83%), mp 212-214° dec. Recrystallization from 95% methanol afforded pure material: mp 213-215° dec; $[\alpha]_D^{22} +44 \pm 2^\circ$ (c 0.5, water).

**Anal.** Calcd for C$_{24}$H$_{22}$N$_4$O$_8$P: C, 54.13; H, 5.49; N, 10.52.
Found: C, 54.33; H, 5.61; N, 10.24.

3. 1-(2-Amino-2-deoxy-β-D-glucopyranosyl)cytosine (III)

1-{2-N-[bis(benzyloxy)phosphinyl]amino-2-deoxy-β-D-glucopyranosyl}-cytosine (IIc, 1.0 g) was dissolved in 92% aqueous dioxane (100 ml) and 0.6 g of 10% palladium-charcoal catalyst added. The mixture was hydrogenated at 30 psi for 4 hr at room temperature. The mixture was filtered and the catalyst was washed with 120 ml of water. The combined filtrate and washings were concentrated to 20 ml and passed through 15 ml of Dowex 1 (CO$_3^{2-}$) ion-exchange resin. The resin was washed with 100 ml of water and the combined eluate and washings were evaporated to yield 0.28 g of hydrogenated material. Thin layer chromatography, using methanol developer and sulfuric acid indicator, showed a major spot at $R_f$ 0.3 (A) and two minor ones at $R_f$ 0.6 (B) and $R_f$ 0.8. Use of a more polar solvent (higher percentage of water) for hydrogenation yielded more of substance B. The crude product was separated by thin layer chromatography (five 200 X 200 X 1 mm silica gel G plates, methanol as developer, and ultraviolet indication) to give 0.17 g of A (60%), mp 215.5-218° dec, and 0.025 g of B. Substance A (III) was crystallized from methanol-ether-chloroform and dried: mp 217-219°
dec with swelling at 170-190°; \([\alpha]_D^{25} +33 \pm 1° (c 1.2, \text{ water})\); \(\lambda_{\text{max}}^{KBr}
2.9-3.1 (\text{OH, NH}), 6.05, 6.25, 6.6, 6.72 \) (cytosine), 7.2, 7.28, 8.3, 9.3, 11.1, and 12.75 \(\mu\text{m}\); \(\lambda_{\text{max}}^{0.1 \text{ N HCl}} = 212\) (\(c 9,600\)) and 275 nm (\(c 12,300\)); \([\alpha]_D^{25} +51.5 \pm 2° \) (as sulfate, \(c 0.5, \text{ water})\); lit. (27) \([\alpha]_D^{25} +50° \) (\(c 1\), water) and \(\lambda_{\text{max}}^{0.1 \text{ N HCl}} = 210\) and 275.5 nm for the sulfate dihydrate.

**Anal.** Calcd for \(C_{10}H_{16}O_5\): C, 44.12; H, 5.88; N, 20.60.

Founs: C, 43.97; H, 5.81; N, 20.60.

Owing to the small amount available, substance B was not further investigated but its ultraviolet spectrum (0.1 N hydrochloric acid) showed one band at 208 nm with no absorption above 210 nm.

4. 2-Trifluoroacetamido-2-deoxy-D-glucose (V)

To a suspension of 2-amino-2-deoxy-D-glucose hydrochloride (IV, 10 g) in anhydrous methanol (50 ml) was added an equivalent amount of sodium methoxide in methanol (1.06 g of sodium dissolved in 10 ml of methanol). The mixture was swirled until only a small residue (sodium chloride) remained. S-Ethyl trifluorothioacetate (10 g) was added and the mixture was allowed to stand at room temperature for 24 hr. The solution was concentrated to a solid residue which was extracted with hot acetone (300 ml) with insoluble material being discarded. After cooling the acetone extract to room temperature, ether (200 ml) was added and the mixture refrigerated overnight. The white crystalline solid which formed was recrystallized from acetone-ether giving 9.3 g (73%) of 2-deoxy-2-trifluoroacetamido-D-glucose (Va); mp 193-195° dec; \([\alpha]_D^{22} +12 \pm 1° \) (initial, extrapolated) \(\rightarrow +15 \pm 1° \) (final, \(c 2.5, \text{ water})\).
A nal. Calcd f or \( \text{C}_{8}\text{H}_{12}\text{F}_{3}\text{NO}_{6} \): C, 34.92; H, 4.40; N, 5.09. Found: C, 35.04; H, 4.70; N, 5.37.

Four additional recrystallizations from acetone gave the \( \beta\)-D anomer (\( \text{Vb} \)): mp 197-198° dec; \([\alpha]^{22}_D \) -23 ± 2° (initial, extrapolated)  

\[ +15 \pm 1° \] (final, \( \alpha 1.5 \), water); \( \lambda_{\text{KBr max}} \) 2.9-3.1 (OH, NH), 5.85 (H-trifluoroacetyl carbonyl), 6.4 (NH), 8.6 (CF), 7.35, 7.6, 7.8, 8.23, 8.45, 9.08, 9.28, 9.6, 9.32, 10.1, 11.1, 11.32, 11.44, and 13.64µm; X-ray powder diffraction data 10.72 µ, 9.41 µw, 6.81 µ, 5.32 vs (2) 5.00 µ, 4.60 vs (3), 4.23 s, 3.95 vs (1), 3.75 µ, 3.68 s, 3.49 vs, 3.19 µ, 3.08 µ, 2.93 µ, 2.84 vs, 2.64 s, 2.50 µ, 2.37 µ, 2.31 vs, 2.21 µ, 2.08 s, 1.99 µ, 1.93 µ, 1.86 µw, 1.77 µ, and 1.66 µ.

A nal. Calcd f or \( \text{C}_{8}\text{H}_{12}\text{F}_{3}\text{NO}_{6} \): C, 34.92; H, 4.40; N, 5.09. Found: C, 34.55; H, 4.29; N, 5.13.

The physical constants of this compound were unchanged by further recrystallization.

5. 2-Deoxy-2-trifluoroacetamido-D-galactose (\( \text{X} \))

A suspension of 2-amino-2-deoxy-D-galactose hydrochloride (\( \text{IX} \), 5 g) in methanol (25 ml) was treated with sodium (0.53 g) in methanol (10 ml) and \( \delta \)-ethyl trifluoroacetate (5 g) by the procedure described in the preceding experiment. The yield after recrystallization of the crude product from acetone-ether was 4.8 g (76%) of 2-deoxy-2-trifluoroacetamido-D-galactose (\( \text{Xa} \)): mp 184-186° dec; \([\alpha]^{21}_D \) +68 ± 2° (initial, extrapolated)  

\[ +59 \pm 1° \] (final, \( \alpha 2.9 \), water).

A nal. Calcd f or \( \text{C}_{8}\text{H}_{12}\text{F}_{3}\text{NO}_{6} \): C, 34.92; H, 4.40; N, 5.09. Found: C, 34.88; H, 4.65; N, 4.88.

Four additional recrystallizations from acetone gave the \( \alpha\)-D
anomer (Xb) mp 192-193° dec; \( [\alpha]_{D}^{22} +108 \pm 2^\circ \) (initial, extrapolated)

\[ \rightarrow +60 \pm 1.5^\circ \] (final, c 2.0, water); \( \lambda^\text{KBr}_{\text{max}} \) 3.0-3.1 (NH, OH) 5.9

(trifluoroacetyl carbonyl), 6.42 (NH), 8.62 (CF), 7.42, 7.68, 7.9, 8.28, 8.35, 8.8, 9.03, 9.18, 9.44, 9.56, 9.7, 9.98, 10.28, 10.6, 11.42, 11.75, 12.43, and 13.7 \( \mu \)m; X-ray powder diffraction data 11.26 vw, 9.88 vw, 8.85 vv, 6.63 m, 5.56 s, 5.10 s (2), 4.77 v, 4.50 s (3), 3.96 s, 3.70 s (2), 3.50 m, 3.31 n, 3.16 m, 3.01 n, 2.84 w, 2.68 v, 2.49 v, 2.39 vv, 2.30 m, 2.22 v, and 2.06 vv.

**Anal.** Calcd for \( \text{C}_{12}\text{H}_{17}\text{F}_{3}\text{NO}_{4} \): C, 34.92; H, 4.40; N, 5.09. Found: C, 34.79; H, 4.69; N, 4.76.

The physical constants of this compound were unchanged by further recrystallization.

6. 1,3,4,6-Tetra-O-acetyl-2-deoxy-2-trifluoroacetamido-\( \alpha \)- and -\( \beta \)-D-glucopyranose (VI and VII)

2-Deoxy-2-trifluoroacetamido-D-glucose (Va, 2.0 g) was dissolved in a pre-cooled (0°) mixture of pyridine (16 ml) and acetic anhydride (9 ml) and the solution kept at room temperature overnight. The solution was poured into ice and water (30 ml) and the mixture extracted with dichloromethane (80 ml). The dichloromethane extract was washed with 1 N hydrochloric acid (until the acid was no longer neutralized), 15% aqueous sodium bicarbonate, and water, then dried over anhydrous sodium sulfate. Evaporation of the solvent yielded a clear syrup which, on thin layer chromatography using ether as developer, showed two major components (\( R_f \) 0.47 and 0.57). The crude product was dissolved in warm (40-50°) methanol (30 ml) and water added until cloudiness just appeared then warmed until the solution became clear. Crys-
tallization ensued immediately upon cooling. The mixture was kept at room temperature for 2 hr then refrigerated overnight. The white crystalline material which formed was re-crystallized from methanol-water to give 1.0 g (31%) of 1,3,4,6-tetra-α-acetyl-2-deoxy-2-trifluoroacetamido-β-D-glucopyranose (VII); mp and mmp with authentic material (29, 30) 166.5-168°; \([a]^{25}_D -12 \pm 1°\) (c 3.4, chloroform); lit. (29, 30) mp 167° \([a]^{22}_D -13°\) (c 2.4, chloroform). X-Ray powder diffraction data were identical to those for the compound prepared previously in this laboratory (29, 30).

This compound was homogeneous by thin layer chromatography using ether as developer and corresponded to the faster moving component of the crude product (Rf 0.57).

The mother liquors from the two crystallizations of VII were evaporated to yield a clear syrupy residue which, on thin layer chromatography using ether as developer, showed a major component (Rf 0.48) and a minor component (Rf 0.56). These two components were isolated by resolution on 24 chromatoplates (200 X 200 X 1.25 mm) with ether as developer and indication by iodine vapor. The two zones were removed and extracted with acetone. Each acetone extract was evaporated and the residues dissolved in dichloromethane. The resulting solutions were washed with 15% aqueous potassium iodide and water, dried over anhydrous sodium sulfate, and the solvent evaporated. The residue from the faster moving zone (Rf 0.56) was crystallized from methanol-water to yield an additional 0.45 g (14%) of VII; mp and mmp 166.5-168°; \([a]^{23}_D -11.5 \pm 2°\) (c 1.5, chloroform).

The material from the slower moving zone (Rf 0.48) was crystallized from ether-hexane to give 1.3 g (40%) of 1,3,4,6-tetra-α-acetyl-2-
deoxy-2-trifluoroacetamido-α-D-glucopyranose (VI): mp 125-126.5°; 
$\beta_{D}^{24} +70 \pm 1 ^\circ$ (c 4.7, chloroform); $\lambda_{\text{KBr}}^\text{max} 3.0$ (NH), 5.7 (Q-acetyl carbonyl), 5.83 (N-trifluoroacetyl carbonyl), 6.4 (NH), 8.1-8.25 (ester), 8.55 (CF), 6.9, 7.0, 7.3, 8.9, 9.4, 9.76, 10.4, 10.62, 10.8, 11.1-11.2, 11.5, 12.95, and 13.7 μm; X-ray powder diffraction data 16.37 w, 10.53 s, 9.46 vs (1), 8.21 s, 7.06 m, 6.08 m, 5.49 w, 5.19 s, 4.82 vs (3), 4.66 m, 4.44 v, 4.29 vs (3), 4.03 v, 3.75 vs (2), 3.52 s, 3.45 s, 3.25 w, 3.02 w, 2.92 m, 2.79 v, 2.64 v, 2.52 m, 2.34 v, 2.22 w, 2.17 v, 2.07 v, and 1.90 v.

Anal. Calcd for C$_{16}$H$_{20}$F$_3$NO$_{10}$: C, 43.35; H, 4.55; N, 3.16.

Found: C, 43.19; H, 4.52; N, 3.49.

This compound was homogeneous by thin layer chromatography using ether as developer.

7. 1,3,4,6-Tetra-Q-acetyl-2-deoxy-2-trifluoroacetamido-α- and -β-D-galactopyranose (XI and XII)

2-Deoxy-2-trifluoroacetamido-D-galactose (Xa, 2.0 g) was acetylated with pyridine (16 ml) and acetic anhydride (9 ml) by the procedure described in the preceding experiment. The crude syrupy product showed two principal components (R$_{f}$ 0.43 and 0.56) on thin layer chromatography using ether as developer. Three crystallizations from methanol-water gave 1,3,4,6-tetra-Q-acetyl-2-deoxy-2-trifluoroacetamido-α-D-galactopyranose (XI) as a white crystalline solid, yield 0.97 g (30%): mp 155-156.5°; $\beta_{D}^{23} +84 \pm 1 ^\circ$ (c 2.1, chloroform); $\lambda_{\text{KBr}}^\text{max} 3.05$ (NH), 5.7 (Q-acetyl carbonyl), 5.85 (N-trifluoroacetyl carbonyl), 6.4 (NH), 8.1-8.3 (ester), 8.68 (CF), 7.32, 9.15, 9.34, 9.6, 9.9, 10.7, 11.1, 11.64, 12.2, 13.1, and 13.8 μm; X-ray powder diffraction data 7.54 vs (1),
6.33 s, 5.85 m, 5.54 s, 5.01 s, 4.63 m, 4.32 vs (2), 4.10 s, 3.96 s, 3.79 v, 3.66 v, 3.51 s (3), 3.41 w, 3.34 vw, 3.05 m, 2.91 w, 2.84 m, 2.77 vw, 2.59 vw, 2.51 m, 2.45 vw, 2.31 v, 2.23 vw, 2.17 m, and 2.11 v.

Anal. Calcd for C_{16}H_{20}O_{3}NO_{10}: C, 43.35; H, 4.55; N, 3.16. Found: C, 43.29; H, 4.40; N, 2.95.

This compound was homogeneous by thin layer chromatography using ether as developer and corresponded to the slower moving component of the crude product (R_f 0.43).

The mother liquors from the three crystallizations of XI were evaporated to yield a syrupy residue which, on thin layer chromatography using ether as developer, showed a major component (R_f 0.57) and a minor component (R_f 0.41). These two components were isolated by resolution on 24 chromatoplates (200 X 200 X 1.25 mm) by the procedure described in the preceding experiment. Crystallization of the material obtained from the slower moving zone (R_f 0.41) from methanol-water yielded an additional 0.52 g (16%) of XI: mp and mmp 155-156.5°; [a]_D^{22} +84 ± 1° (c 2.2, chloroform).

The material obtained from the faster moving zone (R_f 0.57) was crystallized from ether-hexane to give 1.2 g (37%) of 1,3,4,6-tetra-2-bacetyl-2-deoxy-2-trifluoroacetamido-β-D-galactopyranose (XII): mp 130-131°; [a]_D^{21} +11 ± 1° (c 3.1, chloroform); λ_{max} KBr 3.06 (NH), 5.7 (C=O carbonyl) 5.8 (N-trifluoroacetamido carbonyl), 6.36 (NH), 8.1-8.3 (ester), 8.65 (CF), 7.33, 8.45, 9.18, 9.66, 10.46, 11.2, 11.7, 13.52, and 13.9 μm; X-ray powder diffraction data 13.05 m, 7.90 vs (1), 6.25 s, 5.71 s, 5.40 w, 5.17 s (3), 4.80 s, 4.54 m, 4.10 vs (2), 3.83 m, 3.70 m, 3.60 m, 3.43 m, 3.28 s, 3.10 s, 2.98 w, 2.90 m, 2.80 w, 2.70 m, 2.59 m, 2.50 w, 2.44 m, 2.29 m, 2.24 m, 2.18 w, and 2.08 m.
Anal. Calcd for C$_{16}$H$_{20}$F$_3$NO$_{10}$: C, 43.35; H, 4.55; N, 3.16. Found: C, 43.52; H, 4.58; N, 3.57.

This compound was homogeneous by thin layer chromatography using ether as developer.

8. Preparation of 3,4,6-Tri-O-acetyl-2-trifluoroacetamido-\textalpha-D-glucopyranosyl bromide (VIII)

(A) 1,3,4,6-Tetra-O-acetyl-2-trifluoroacetamido-\textalpha-D-glucopyranose (VI, 2.0 g) was moistened with chloroform (1.0 ml) and acetic acid nearly saturated at 0° with hydrogen bromide (2.0 ml) was added. The mixture was allowed to stand at room temperature in a glass stoppered flask (protected from moisture) for 2 hr then dissolved in dichloromethane (30 ml). The resulting solution was washed with cold 20% aqueous sodium acetate and water, then dried over anhydrous magnesium sulfate. Evaporation of the solvent gave a syrupy residue which was crystallized from ether-hexane, yield 2.0 g (96%): mp 96-97°; [\alpha]$_D^{20}$ +125 ± 1° (c 2.7, chloroform); lit. (190) mp 95-97°, lit. (29, 30) mp 96°; [\alpha]$_D^{21}$ +126° (c 2.92, chloroform).

(B) 1,3,4,6-Tetra-O-acetyl-2-deoxy-2-trifluoroacetamido-\textbeta-D-glucopyranose (VII, 1.0 g) was reacted with acetic acid nearly saturated at 0° with hydrogen bromide by the procedure described in the preceding experiment. The yield after crystallization from ether-hexane was 1.0 g (96%): mp and mmp 95-97°; [\alpha]$_D^{21}$ +126 ± 1° (c 1.9, chloroform).

(C) Since VIII could be obtained from VI and VII, its preparation from a mixture of VI and VII was undertaken to avoid the isolation of the individual anomeric acetates. 2-Deoxy-2-trifluoroacetamido-\textbeta-D-glucose (Va, 0.8 g) was acetylated with a mixture of pyridine (6.5 ml)
and acetic anhydride (3.5) by the procedure described for the preparation of VI and VII. The crude syrupy product was crystallized from ether-hexane giving a white crystalline material, yield 1.1 g (85%), mp 116-121°C. Thin layer chromatography of the product using ether as developer showed two components (Rf 0.45 and 0.56) which presumably were 1,3,4,6-tetra-O-acetyl-2-deoxy-2-trifluoroacetamido-α-D-glucopyranose (VI) and the corresponding β-D anomer (VII), the two anomers having co-crystallized.

The material was reacted with acetic acid nearly saturated at 0°C with hydrogen bromide (1.1 ml) following the procedure used in the preceding two experiments. Crystallization of the crude product from ether-hexane gave VIII, yield 1.08 g (94%): mp and mmp 96-97°C; [α]_D^21 +127 ± 2°C (c 1.4, chloroform).

9. 3,4,6-Tri-O-acetyl-2-deoxy-2-trifluoroacetamido-
   α-D-galactopyranosyl bromide (XIII)

(A) 1,3,4,6-Tetra-O-acetyl-2-deoxy-2-trifluoroacetamido-α-D-
   galactopyranose (XI, 1.0 g) was reacted with acetic acid nearly saturated at 0°C with hydrogen bromide by the procedure used in the preceding experiment for the preparation of VIII. A clear syrupy product was obtained which formed a solid foam upon removal of the last traces of solvent under reduced pressure (water aspirator) in a vacuum desiccator, yield 1.02 g (97%): mp 60-62°C; [α]_D^22 +146 ± 1°C (c 1.5, chloroform);

\[ \lambda_{\text{max}}^{\text{KBr}} \]
3.05 (NH), 5.7-5.8 (O-acetyl and N-trifluoroacetyl carbonyl), 6.45 (NH), 8.1-8.3 (ester), 8.65 (CF), 7.3, 9.2, 10.55, 11.1, 11.8, 12.9, and 13.7 μm.
Anal. Calcd for C_{14}H_{17}F_{3}O_{3}: C, 36.22; H, 3.69; N, 3.02. Found: C, 36.22; H, 3.90; N, 2.97.

The compound was homogeneous by thin layer chromatography using ether-hexane (2:1) as developer. Attempts to crystallize the product were unsuccessful.

(B) Treatment of 1,3,4,6-tetra-2-acetyl-2-deoxy-2-trifluoroacetanido-β-D-galactopyranose (XII, 0.50 g) with acetic acid nearly saturated at 0° with hydrogen bromide (0.50 ml) by the procedure used in the preceding experiment gave 0.49 g (94%) of XIII: m.p. 60-63°; [α]_{D}^{20} +144° ± 2° (c 1.1, chloroform).

(C) Compound XIII was also prepared from a mixture of XI and XII thus avoiding the isolation of the individual anomeric acetates.

2-Deoxy-2-trifluoroacetanido-D-galactose (Xa, 0.60 g) was acetylated with a mixture of pyridine (4.5 ml) and acetic anhydride (3.0 ml) by the procedure previously described for the preparation of VI and VII. The crude syrupy product was dissolved in methanol (30 ml), water (30 ml) was added, and the solution was concentrated to ½ volume. After refrigeration overnight, a white crystalline material formed, yield 0.58 g (60%), m.p. 128-132°. Further concentration of the mother liquor to about 15 ml followed by refrigeration overnight produced a second crop of crystalline material, yield 0.20 g (21%): m.p. 121-126°. Treatment of the combined material (0.78 g) with acetic acid nearly saturated at 0° with hydrogen bromide (0.8 ml) by the procedure used in the preceding experiment produced XIII, yield 0.76 g (93%): m.p. 59-62°; [α]_{D}^{22} +143° ± 2° (c 1.0, chloroform).
10. 1-(3,4,6-Tri-O-acetyl-2-deoxy-2-trifluoroacetamido-β-D-glucopyranosyl)cytosine (XIV)

(A) To a solution of 3,4,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-α-D-glucopyranosyl bromide (VIII, 1.0 g) in benzene (40 ml) was added, with stirring, bis(trimethylsilyl)cytosine (119, 123) (1.0 g). The mixture was refluxed, with stirring, for 4 hr after which it was cooled to room temperature. The white precipitate which formed was collected by filtration and washed with benzene (30 ml). The material was added to 80% aqueous ethanol (40 ml) containing sodium bicarbonate (0.5 g) and the mixture heated at 60° for 15 min. The solution was evaporated to dryness and the residue extracted with chloroform (120 ml). Evaporation of the dried (sodium sulfate) extracted to dryness yielded a pale yellow glass (0.60 g). The crude product was crystallized slowly from moist methanol-chloroform-isopropyl ether to give the crystalline monohydrate, yield 0.45 g (41%): mp 224-226°; X-ray powder diffraction data 12.11 s (1), 10.11 m, 9.89 m, 8.08 m, 7.44 m, 6.86 v, 6.07 w, 5.72 v, 5.37 s (2), 5.00 w, 4.73 w, 4.51 m, 4.36 s (2), 4.25 v, 3.99 v, 3.88 s (2), 3.75 v, 3.61 m, and 3.49 v.

Anal. Calcd for C_{18}H_{21}F_{3}N_{4}O_{9}·H_{2}O: C, 42.19; H, 4.49; N, 10.93. Found: C, 42.01; H, 4.85; N, 11.11.

Drying of this compound for 4 hr at 110° under diminished pressure (0.4 mm) gave the anhydrous form as a white crystalline solid: mp 226-228°; [α]^{25}_{D} +6 ± 1° (c 3.2, chloroform); λ_{\text{max}}^{KBr} 3.05 (NH₂), 5.7-5.8 (O-acetyl and N-trifluoroacetyl carbonyl), 6.05, 6.4, 6.7 (NH, cytosine), 8.0-8.3 (ester), 8.6 (OF), 7.3, 8.45, 9.0, 9.2, 9.55, 10.38, 10.75, 11.5, 11.75, 12.68, and 13.7 μm; λ_{\text{max}}^{EtOH} 206 (ε 14,800), 245 (ε 7,920),
and 270 nm (shoulder, ε 8,130); X-ray powder diffraction data 11.41 m,
9.51 m, 7.69 s, 7.20 m, 6.73 m, 6.22 v, 5.75 w, 5.37 s (1), 5.04 m,
4.60 s (2), 4.23 s, 4.12 s (3), 3.75 v, 3.61 s, 3.41 m, 3.30 w, 3.16 w,
2.96 w, and 2.89 w.

Anal. Calcd for C₄₃H₂₁F₃N₄O₉: C, 43.73; H, 4.28; N, 11.33.
Found: C, 43.66; H, 4.41; N, 11.50.

This compound was homogeneous by thin layer chromatography using
acetone-chloroform (5:2) or ethyl acetate-methanol (10:1) as developer.
Examination of the mother liquors from the crystallization of XIV by
thin layer chromatography using acetone-chloroform (5:2) or ethyl
acetate-methanol (10:1) as developer gave no evidence of a second
anomer.

(B) 3',4',6-Tri-O-acetyl-2-deoxy-2-trifluoroacetamido-α-D-glucopyranosyl bromide (VIII, 1.0 g) was dissolved in chloroform (20 ml)
and bis(trimethylsilyl)cytosine (1.5 g) added with stirring. Stirring
was continued for several min until a uniform mixture was obtained. The
solvent was evaporated and the resulting syrupy residue heated at 130-
140° in an oil bath for 20 min under diminished pressure (water aspira-
tor). After cooling to room temperature, the product was added to 80%
aqueous methanol (40 ml) containing sodium bicarbonate (0.5 g) and
the mixture heated at 60° for 15 min. Evaporation of the solvent gave
an amber colored residue which was extracted with chloroform (100 ml).
The dried (sodium sulfate) extract was evaporated to a small volume
(10 ml) and ether (60 ml) added. The precipitate which formed was
filtered, washed with ether (30 ml), and crystallized slowly from
moist methanol-chloroform-isopropyl ether to give XIV as the crys-
talline hydrate, yield 0.73 g (66%), mp and mmp with the compound pre-
pared by the procedure described in part A 124-126°. X-Ray powder
diffraction data were identical to those for the compound prepared
by the procedure described in part A.

11. Deblocking of 1-(3,4,6-Tri-O-acetyl-2-deoxy-2-
trifluoroacetamido-β-D-glucopyranosyl)cytosine (XIV)

1-(3,4,6-Tri-β-acetyl-2-deoxy-2-trifluoroacetamido-β-D-glucopyranosyl)cytosine (XIV, 0.30 g) was dissolved in methanol saturated at 0°
with ammonia (40 ml) and the solution allowed to remain at room tempera-
ture for 6 days. The solution was evaporated to a small volume (10 ml)
and ether (80 ml) added. The resulting flocculent precipitate was
filtered, washed with ether, and crystallized from methanol-chloroform-
ether to give 1-(2-amino-2-deoxy-β-D-glucopyranosyl)cytosine (III),
yield 0.15 g (91%); mp and mmp with III prepared from 1-[2-N-[(bis-
(benzyloxy)phosphinyl)amino-2-deoxy-β-D-glucopyranosyl]cytosine (IIc)
217-219° dec with swelling above 172°; [α]$_D ^{23} +32 ± 1.5°$ (c 1.4, water).

12. 6-Benzamido-9-(3,4,6-tri-O-acetyl-2-deoxy-2-
trifluoroacetamido-α- and -β-D-glucopyranosyl)purine (XVa and XVIIa)

A mixture of 6-benzamido-9-chloromercuripurine (109) (8.0 g),
cadmium carbonate (2.0 g), and celite (233) (2.5 g) in toluene (75 ml)

(233) No. 225, a siliceous filter aid, Johns-Manville, Inc.,
New York, N. Y.

was azeotropically dried by distillation of approximately 1/3 of the
solvent. To the hot suspension was added, with stirring, 3,4,6-tri-
124.

- O-acetyl-2-deoxy-2-trifluoroacetamido-\(\alpha\)-D-glucopyranosyl bromide (VIII, 2.6 g). The mixture was refluxed for 3 hr, with stirring, then kept at room temperature overnight. The mixture was poured into cold (0°) petroleum ether (bp 30-60°, 100 ml). The precipitate which formed was collected by filtration and extracted with chloroform (300 ml). The chloroform solution was washed with 30% aqueous potassium iodide and with water. The dried (sodium sulfate) solution was concentrated to a pale amber glass (1.65 g) which, on thin layer chromatography using chloroform-acetone (5:2) as developer, showed a major component (\(R_f\) 0.35) and two minor components (\(R_f\) 0.6 and 0.8). The crude product was resolved by preparative thin layer chromatography on 24 chromatoplates (200 X 200 X 1.25 mm) with chloroform-acetone (5:2) as developer and indication by uv light. The two slower moving zones (\(R_f\) 0.35 and 0.6) were removed and extracted with acetone. Evaporation of the extract from the faster of these two zones (\(R_f\) 0.6) gave a clear glass (0.29 g) which was crystallized from chloroform-isopropyl ether to give a gelatinous mixture which yielded 6-benzamido-9-(3,4,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido)-\(\alpha\)-D-glucopyranosylpurine (XVA) as a white crystalline solid upon suction filtration, yield 0.23 g (6.6%): mp 169-172°; [\(\alpha\)]_D^20 +105 ± 2.5° (c 0.7, chloroform); \(\lambda_{\text{max}}^{\text{KBR}}\) 3.1 (NH), 5.75 (O-acetyl and \(N\)-trifluoroacetamide carbonyl), 6.2, 6.35, 6.66, 6.9 (aryl C=C, purine, NH), 8.1-8.3 (ester), 8.6 (CF), 14.1 (substituted benzene), 7.36, 7.55, 9.15, 9.6, 10.3, 11.2, 12.52, and 13.6 \(\mu m\); \(\lambda_{\text{max}}^{\text{EtOH}}\) 211 (c 21,000), 234 (c 13,500), and 282 nm (c 19,500); X-ray powder diffraction data 13.81 s (1), 11.95 m, 10.43 s (3), 9.69 \(\mu m\), 8.61 s, 7.45 m, 7.01 \(\mu m\), 6.50 m, 6.05 m, 5.68 m, 5.28 w, 5.01 w, 4.82 m, 4.60 m, 4.23 w, 3.84 w, 3.60 s (2), 3.32 m, 3.05 w, 2.82 w, and 2.64 w.
This compound was homogeneous by thin layer chromatography using chloroform-acetone (3:1) as developer.

Evaporation of the extract from the slower zone (Rf 0.35) gave a clear glass (1.04 g). Attempted crystallization from chloroform-isopropyl ether gave 6-benzamido-9-(3,4,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-β-D-gluco.pyranosyl)purine (XVIIa) as a white amorphous (234) solid, yield 0.90 g (26%): mp 148-153° with softening above 133°; [α]$_D^{21}$ +50 ± 1° (c 2.6, chloroform); λ$_{max}^{KBr}$ 3.05 (NH), 5.7 (O-acetyl and N-trifluoroacetyl carbonyl), 6.2, 6.32, 6.65, 6.75, 6.9 (aryl C=C, purine, NH), 8.1-8.3 (ester), 8.6 (CF), 14.1 (substituted benzene), 7.32, 8.7, 9.3, 10.3, 11.2, 12.2, 12.5, and 13.2 μm; λ$_{max}^{EtOH}$ 210 (ε 21,200), 234 (ε 12,500), and 280 nm (ε 19,700).

This compound was concluded to be amorphous on the basis of a blank or foggy X-ray powder diffraction pattern.

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Anal. Calcd for C$_{26}$H$_{25}$F$_3$N$_6$O$_9$: C, 50.16; H, 4.05; N, 13.50.

Found: C, 50.19; H, 4.14; N, 13.70.
13. \(9-(3,4,6\text{-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-\(\alpha\)-D-glucopyranosyl})\)adenine picrate (XV\(\text{b}\))

To a solution of \(6\)-benzamido-\(9-(3,4,6\text{-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-\(\alpha\)-D-glucopyranosyl})\)purine (XV\(\text{a}\), 60 mg) in 2-propanol (10 ml) was added a solution of picric acid (25 mg) in methanol (5 ml). The solution was concentrated to about \(1/3\) volume by boiling (20 min), allowed to cool slowly to room temperature, then refrigerated overnight. The yellow crystalline solid which separated was filtered and washed with cold hexane, yield 61 mg (84%), mp 210-213° dec. Further recrystallizations from 2-propanol-methanol-petroleum ether (bp 60-110°) afforded pure material: mp 216-217° dec; \([\alpha]_D^{22} +83 \pm 2°\) (c 0.6, acetone); \(\lambda_{\text{max}}^{\text{KBr}}\) 3.1-3.3 (NH, \(\text{NH}_2^+\)), 5.75 (\(\text{O-acetyl}\) and \(\text{N-trifluoroacetyl carbonyl}\)), 5.92, 6.2, 6.4, 6.7 (aryl C=C, purine, NH), 6.5, 7.6 (NO\(_2\)), 8.1-8.25 (ester), 8.6 (CF), 14.0-14.3 (substituted benzene), 7.05, 7.35, 9.6, 10.96, 12.66, and 13.4 \(\mu\)m; X-ray powder diffraction data 11.63 s, 10.34 w, 8.23 vs (1), 6.94 m, 6.28 m, 5.79 w, 5.45 m, 5.25 w, 4.81 m, 4.50 m, 4.32 m, 4.23 m, 3.99 s (2), 3.81 w, 3.64 w, 3.47 m, 3.35 s (3), 3.20 wv, and 3.02 m.


Found: C, 40.39; H, 3.52; N, 16.68.

14. \(9-(3,4,6\text{-Tri-O-acetyl-2-deoxy-2-trifluoroacetamido-\(\beta\)-D-glucopyranosyl})\)adenine picrate (XV\(\text{b}\))

A solution of \(6\)-benzamido-\(9-(3,4,6\text{-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-\(\beta\)-D-glucopyranosyl})\)purine (XV\(\text{a}\), 200 mg) in 2-propanol (15 ml) was treated with picric acid (80 mg) in methanol (8 ml).
by the procedure described in the preceding experiment, yield 220 mg
(92%), mp 207-210° dec. Recrystallization from 2-propanol afforded
pure material: 212-214° dec; \( [\alpha]_D^{27} -39 \pm 1^\circ \) (c 1.9, acetone); \( \lambda_{max} \)
3.0-3.25 (NH, NH\(^+_3\)), 5.72 (Q-acetyl and N-trifluoroacetyl carbonyl),
5.92, 6.3, 6.35, 6.68 (aryl C=O, purine, NH), 6.5, 7.6 (NO\(_2\)), 8.1-8.3
(ester), 8.6 (CF), 14.1 (substituted benzene), 7.04, 7.35, 9.25, 9.6,
11.0, 12.25, 12.65, and 13.45 \( \mu \)m; X-ray powder diffraction data 12.63 s
(1), 10.28 m, 9.07 m, 7.05 m, 6.44 m, 6.01 w, 5.47 s (3), 5.00 s,
4.50 s, 4.22 s (2), 4.01 w, 3.83 w, 3.69 w, 3.52 s, 3.36 m, 3.25 w,
3.16 w, and 3.10 w.

Found: C, 40.13; H, 3.16; N, 16.79.

15. 9-(2-Amino-2-deoxy-\(\alpha\)-D-glucopyranosyl)adenine (XVI)

6-Benzamido-9-(3,4,6-tri-Q-acetyl-2-deoxy-2-trifluoroacetamido-\(\alpha\-
D-glucopyranosyl)purine (XVA, 200 mg) was dissolved in methanol nearly
saturated at 0° with ammonia (40 ml). The solution was kept at room
temperature for 7 days after which it was concentrated to a small volume
(5 ml) and excess ether (50 ml) added. The resulting white flocculent
precipitate was filtered, washed with ether, and recrystallized from
methanol-ethanol, yield 85 mg (89%); mp and mmp with authentic material
(31) 242-244° dec; \( [\alpha]_D^{21} +84 \pm 2^\circ \) (c 1.5, water); lit. (31) mp 242-244°
dec, \( [\alpha]_D^{22} +83 \pm 6^\circ \) (c 0.2, water).
16. 9-(2-Amino-2-deoxy-β-D-glucopyranosyl)adenine (XVIII)

6-Benzamido-9-(3,4,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-β-D-glucopyranosyl)purine (XVIIa, 0.50 g) was treated with methanol nearly saturated at 0° with ammonia (80 ml) by the procedure described in the preceding experiment. The yield after recrystallization from ethanol was 0.205 g (86%); mp and mmp with authentic material (31) 185-188° dec; [α]$_D^{22}$ -17 ± 1° (c 2.3, water); lit. (31) mp 186-188° dec, [α]$_D^{23}$ -17 ± 2° (c 0.2, water).

17. 1-(3,4,6-Tri-O-acetyl-2-deoxy-2-trifluoroacetamido-β-D-galactopyranosyl)cytosine (XIX)

3,4,6-Tri-O-acetyl-2-deoxy-2-trifluoroacetamido-α-D-galactopyranosyl bromide (XIII, 2.0 g) was dissolved in benzene (15 ml) and bis(trimethylsilyl)cytosine (119, 123) (3.0 g) was added. After thorough mixing, the solvent was evaporated and the resulting syrupy residue was heated at 130-140° in an oil bath for 20 min under diminished pressure (water aspirator). After cooling to room temperature, the crude product was added to 80% aqueous ethanol (100 ml) containing sodium bicarbonate (1.0 g) and the mixture heated for 15 min at 60°. The mixture was evaporated to dryness and the residue extracted with chloroform (160 ml). The dried (sodium sulfate) extract was evaporated to a small volume (15 ml) and ether (100 ml) was added. The resulting precipitate was collected by filtration and crystallized from moist methanol-isopropyl ether to give the crystalline monohydrate, yield 1.63 g (74%): mp 168-172° with softening above 155°; X-ray powder diffraction data 13.76 vs (1), 9.99 m, 8.61 vs (3), 7.69 m, 7.04 m,
6.28 vs (2), 5.77 w, 4.96 m, 4.67 s, 4.44 s, 4.26 vw, 4.10 s, 3.91 s, 3.74 s, 3.55 w, 3.42 s, 3.21 w, 3.02 v, 2.80 v, 2.71 vw, 2.60 vw, 2.49 vw, and 2.37 w.

**Anal.** Calcd for C₁₈H₂₁F₃N₄Oₙ·H₂O: C, 42.19; H, 4.49; N, 10.93. Found: C, 41.95; H, 4.69; N, 10.62.

Drying of this compound for 24 hr in a vacuum desiccator gave the anhydrous form as a white amorphous (234) powder: mp 168–172° with softening above 155°; [α]₂₂^22^2^2^2 +24 ± 10 (c 1.3, chloroform); λ_max KBr 3.1 (NH, NH₂), 5.7–5.8 (O-acetyl and N-trifluoroacetyl carbonyl), 6.05, 6.44, 6.6, 6.7 (cytosine, NH), 8.1–8.3 (ester), 8.65 (CF), 7.32, 8.95, 9.25, 9.55, 10.54, 10.8, 11.6, 12.7, and 13.8 μm; λ_EthOH max 206 (ε 17,400), 245 (ε 8,840), and 270 nm (shoulder, ε 8,100).

**Anal.** Calcd for C₁₈H₂₁F₃N₄Oₙ: C, 43.73; H, 4.37; N, 11.33. Found: C, 43.80; H, 4.37; N, 11.14.

This compound was homogeneous by thin layer chromatography using acetone–chloroform (3:1) or ethyl acetate–methanol (10:1) as developer. Examination of the mother liquors from the crystallization of XIX by thin layer chromatography, using acetone–chloroform (5:2) or ethyl acetate–methanol (10:1) as developer gave no evidence of a second anomer.

18. 1-(2-Amino-2-deoxy-β-D-galactopyranosyl)cytosine (XX)

1-(3,4,6-Tri-O-acetyl-2-deoxy-2-trifluoroacetamido-β-D-galactopyranosyl)cytosine (XIX, 0.24 g) was decylated with methanol saturated at 0° with ammonia (50 ml) by the procedure described for the deblocking of XIV. Crystallization of the crude product from methanol gave
a white crystalline material, yield 0.12 g (91%); mp 233-237° with
softening above 170° and swelling above 180°; \([\alpha]_D^{20} +67 \pm 1.5° (\pm 1.5, water); \lambda_{\text{max}}^{\text{KBr}} 2.9-3.1 (\text{OH, NH}_2), 6.05, 6.2, 6.6, 6.75 (cytosine), 7.3, 7.8, 8.35, 9.3, 11.3, and 12.83 \mu\text{m}; \lambda_{\text{max}}^{\text{H}_2\text{O}} 203 (e 15,000), 237 (e 7,750), and 270 nm (e 8,300); \lambda_{\text{max}}^{0.1 \text{ N HCl}} 212 (e 9,100), and 276 nm (e 12,100);

nur spectrum (deuterium oxide), 6 3.18-4.0 (sugar ring protons), 4.70 (solvent), 5.58 (distinct doublet, \(J_{1,2} = 9.4 \text{ Hz, H-1'}\)), 6.07 (doublet, \(J = 7.5 \text{ Hz, H-4}\)), and 7.71 (doublet, \(J = 7.5 \text{ Hz, H-5}\)); X-ray powder
diffraction data 10.46 m, 6.34 s (1), 5.95 v, 5.52 s (2), 5.22 v, 5.02 s, 4.70 s, 4.24 s, 3.84 s (3), 3.60 m, 3.47 s (3), 3.33 m, 3.14 vv, 2.98 v, 2.86 s, 2.76 m, 2.64 m, 2.51 v, 2.36 v, 2.22 vv, 2.15 vv, 2.08 vv, and 1.93 v.

Anal. Calcd for \(\text{C}_{10}\text{H}_{16}\text{N}_4\text{O}_5\): C, 44.11; H, 5.92; N, 20.53. Found: C, 44.38; H, 6.13; N, 20.61.

This compound was homogeneous by thin layer chromatography using
methanol as developer.

19. 1-(3,4,6-Tri-\text{Q}-acetyl-2-deoxy-2-
trifluoroacetamido-\text{D}-galactopyranosyl)thymine (XXI)

3,4,6-Tri-\text{Q}-acetyl-2-deoxy-2-trifluoroacetamido-\text{D}-galactopyranosyl bromide (XIII, 2.5 g) was dissolved in chloroform (20 ml) and
bis(trimethylsilyl)thymine (5.0 g) added. After thorough mixing, the
solvent was evaporated and the resulting residue heated in an oil bath
at 120-130° for 20 min. After cooling to room temperature, the crude
product was added to methanol (100 ml) and the mixture heated at 60°
for 15 min. The solvent was evaporated and the residue extracted with
chloroform (200 ml). The chloroform extract was washed with water, dried over anhydrous sodium sulfate, and the solvent evaporated to yield a pale yellow glass. The crude product was crystallized from chloroform-isopropyl ether by slow evaporation to a small volume giving a white crystalline material, yield 2.2 g (80%): mp 174-179° with softening above 160°; [α]20 $^\text{D} +4.0 \pm 1^\circ$ (c 3.0, chloroform); λ$^{\text{max}}_{\text{Br}}$ 3.1 (in), 5.7-5.8 (O-acetyl and N-trifluoroacetyl carbonyl), 5.9, 6.42, 6.9 (in, thymine), 8.1-8.3 (ester), 8.65 (CF), 7.3, 9.25, 9.55, 10.66, 11.2, 12.7, and 13.68 μm; λ$^{\text{max}}_{\text{EtOH}}$ 212 (ε 10,300) and 262 nm (ε 9,100); X-ray powder diffraction data: 8.76 s (1), 8.47 m, 7.76 m, 6.94 m, 6.15 w, 5.63 m, 4.98 vv, 4.80 m, 4.48 v, 4.33 s, 4.20 s (2), 3.83 s (3), 3.76 v, 3.11 mw, and 2.84 vv.

Anal. Calcd for C$_{19}$H$_2$F$_2$N$_3$O$_{10}$: C, 44.30; H, 4.35; N, 8.25. Found: C, 45.14; H, 4.37; N, 8.54.

This compound was homogeneous by thin layer chromatography using chloroform-acetone (3:2) or ethyl acetate-benzene (2:1) as developer. Examination of the mother liquors from the crystallization of XXI by thin layer chromatography using chloroform-acetone (3:2) or ethyl acetate-benzene (2:1) as developer gave no evidence of a second anomer.

20. 1-(2-amino-2-deoxy-β-D-galactopyranosyl)thymine (XXII)

1-(3,4,6-Tri-O-acetyl-2-deoxy-2-trifluoroacetamido-β-D-galactopyranosyl)thymine (XXI, 1.0 g) was deacetylated with methanol saturated at 0° with ammonia (120 ml) by the procedure described for the deblocking of XIV. Attempted crystallization of the crude product from methanol-ether gave white amorphous (234) material, yield 0.47 g (83%); mp
indefinite: 110-190°; dec pt 243-245°; [a]$_D^{20}$ +49 ± 2° (c 1.5, water); 
$\lambda_{\text{max}}^\text{KBr}$ 2.9-3.0 (OH, H$_2$O), 5.9, 6.8 (thymine), 7.3, 7.82, 9.2, 11.3, 12.8, 
and 13.85 μm; $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ 209 (ε 10,500) and 266 nm (ε 8,870); nmr spectrum 
(deuterium oxide), δ 1.90 (doublet, J = 1.5 Hz, thymine CH$_3$), 3.02- 
4.02 (sugar ring protons), 4.7 (solvent), 5.52 (distinct doublet, 
J$_1,2 = 9.4$ Hz, H-1'), and 7.65 (multiplet, J = 1.5 cps, H-5).

Anal. Caled for C$_{45}$H$_{49}$O$_6$: C, 45.99; H, 5.96; N, 14.63. Found: 
C, 45.73; H, 5.78; N, 14.38.

This compound was homogeneous by thin layer chromatography using 
methanol-ethyl acetate (1:1) as developer.

21. 6-Benzamido-9-(3,4,6-tri-O-acetyl-2-deoxy-2-
trifluoroacetamido-α- and -β-D-galactopyranosyl)purine (XXIIIa and XXIVa)

3,4,6-Tri-O-acetyl-2-deoxy-2-trifluoroacetamido-α-D-galactopyranosyl bromide (XIII, 2.0 g) was condensed with 6-benzamido-9-chloromercuri-
purine (6.0 g) in refluxing toluene (50 ml) in the presence of cadmium 
carbonate (2.0 g) and celite (2.0 g) by the procedure described for the 
preparation of XVa and XVIIa. A pale amber glass (1.6 g) was obtained 
which, on thin layer chromatography using chloroform-acetone (5:2) as 
developer, showed a major component (R$_f$ 0.33) and two minor components 
(R$_f$ 0.6 and 0.8). The crude product was resolved on 24 chromatoplates 
(200 X 200 X 1.25 mm) with chloroform-acetone (5:2) as developer and 
indication by uv light. The two slower moving zones (R$_f$ 0.33 and 0.6) 
were removed and extracted with acetone. Evaporation of the extract 
from the faster of these two zones (R$_f$ 0.6) gave a clear glass (0.31 g) 
which was crystallized from chloroform-isopropyl ether to give a gelati-
nous mixture which yielded 6-benzamido-9-(3,4,6-tri-O-acetyl-2-deoxy-2-
trifluoroacetamido-α-D-galactopyranosyl)purine (XXIIIa) as a white
crystalline solid upon suction filtration, yield 0.25 g (9.3%): mp
140-144° with softening above 130°; [α]$_D^{22}$ +120 ± 3° (c 1.0, chloroform),
λ$_{max}^{EtOH}$ 3.1 (NH), 5.75 (O-acetyl and N-trifluoroacetyl carbonyl), 6.2, 6.35,
6.65, 6.76, 6.9 (aryl C=O, purine, NH), 8.1-8.3 (ester), 3.65 (CF), 14.1
(substituted benzene), 7.32, 7.55, 9.2, 10.55, 11.1, 11.7, and 12.52
μm; λ$_{max}^{EtOH}$ 210 (ε 13,300) and 282 nm (ε 19,500); X-ray powder diffraction
data 13.50 w, 9.82 s (1), 5.17 m, 4.42 m, 3.38 s (2), 3.55 w, and
3.33 s (3).

Anal. Calcd for C$_{26}$H$_{25}$F$_3$N$_5$O$_9$: C, 50.16; H, 4.05; N, 13.50.
Found: C, 50.21; H, 4.42; N, 13.42.

This compound was homogeneous by thin layer chromatography using
chloroform-acetone (3:1) as developer.

Evaporation of the extract from the slower zone (R$_f$ 0.33) gave a
clear glass (0.94 g). Attempted crystallization from chloroform-
isopropyl ether yielded 6-benzamido-9-(3,4,6-tri-O-acetyl-2-deoxy-2-
trifluoroacetamido-β-D-galactopyranosyl)purine (XXIVa) as an amorphous
(234) white solid, yield 0.30 g (30%): mp 150-155° with softening
above 135°; [α]$_D^{19}$ -34 ± 1° (c 2.7, chloroform); λ$_{max}^{KBr}$ 3.05 (NH), 5.72
(O-acetyl and N-trifluoroacetyl carbonyl), 6.2, 6.32, 6.65, 6.9 (aryl
C=O, purine, NH), 8.1-8.3 (ester), 8.65 (CF), 14.1 (substituted benzene),
9.2, 10.5, 10.85, 12.55, and 13.2 μm; λ$_{max}^{EtOH}$ 211 (ε 20,900), 234 (ε 12,600),
and 280 nm (ε 19,900).

Anal. Calcd for C$_{26}$H$_{25}$F$_3$N$_5$O$_9$: C, 50.16; H, 4.05; N, 13.50.
Found: C, 50.21; H, 4.36; N, 13.56.
This compound was homogeneous by thin layer chromatography using chloroform-acetone (3:1) as developer.

22. 9-(3,4,6-Tri-O-acetyl-2-deoxy-2-trifluoroacetamido-α-D-galactopyranosyl)adenine Picrate (XXIIIB)

A solution of 6-benzamido-9-(3,4,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-α-D-galactopyranosyl)purine (XXIIIA, 50 mg) in 2-propanol (8 ml) was treated with picric acid (20 mg) in methanol (4 ml) by the procedure described for preparation of XVb. The yield of crude product was 49 mg (82%): mp 177-182°. Further recrystallizations from 2-propanol-methanol-petroleum ether (bp 60-110°) afforded pure material: mp 181-185°; [a]$_D^{22}$ +108 ± 4° (c 0.3, acetone); λ$_{max}$ $\text{KBr}$ 3.1-3.3 (NH, NH$_3$), 5.76 (O-acetyl and N-trifluoroacetyl carbonyl), 5.92, 6.2, 6.4, 6.7 (aryl C=O, purine, NH), 6.5, 7.6 (NO$_2$), 8.65 (CF), 14.1-14.2 (substituted benzene), 7.05, 7.36, 9.5, 11.0, 12.7, and 13.5 μm; X-ray powder diffraction data 10.72 w, 9.51 s (2), 7.53 v, 6.73 w, 5.28 w, 4.63 m, 4.34 m, 3.93 s (t), and 3.50 m (3).

Anal. Calcd for C$_{25}$H$_{24}$F$_3$N$_2$O$_4$: C, 40.17; H, 3.24; N, 16.86.

Found: C, 40.01; H, 3.35; N, 16.64.

23. 9-(3,4,6-Tri-O-acetyl-2-deoxy-2-trifluoroacetamido-β-D-galactopyranosyl)adenine Picrate (XXIVb)

A solution of 6-benzamido-9-(3,4,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-β-D-galactopyranosyl)purine (XXIVA, 180 mg) in propanol (12 ml) was treated with picric acid (75 mg) in methanol (7 ml) by the procedure described for the preparation of XVb. The yield of crude product
was 190 mg (83%), mp 203-205° dec. Recrystallization from 2-propanol afforded pure material mp 206-207° dec; [α]$_D^{19}$ -37 ± 1.5° (c 1.3, acetone); λ$_{KBr}^{max}$ 3.05-3.3 (NH, NH$_2$), 5.75 (Q-acetyl and Q-trifluoroacetyl carbonyl), 5.92, 6.2, 6.4, 6.7 (aryl C=O, purine, NH), 6.5, 7.6 (NO$_2$), 8.2 (ester), 8.6 (CF), 13.9-14.05 (substituted benzene), 7.05, 7.36, 9.25, 9.55, 10.85, 12.7, and 13.5 μm; X-ray powder diffraction data

11.05 v, 8.93 s (1), 7.35 wv, 7.28 s (3), 6.51 w, 5.88 w, 5.44 w, 5.05 w, 4.81 v, 4.59 w, 4.47 s, 4.36 v, 4.19 w, 3.99 wv, 3.74 wv, 3.60 w, 3.40 s (2), 3.28 w, 3.21 v, 3.09 w, 2.91 wv, 2.83 wv, and 2.73 v.


24. 9-(2-Amino-2-deoxy-a-D-galactopyranosyl)adenine (XXIIIc)

6-Benzamido-9-(3,4,6-tri-Q-acetyl-2-deoxy-2-trifluoroacetamido-a-D-galactopyranosyl)purine (XXIIIa, 170 mg) was deacylated with methanol nearly saturated at 0° with ammonia (40 ml) by the procedure described for the preparation of XVI. Attempted crystallization of the crude product from methanol–ether gave a white amorphous (234) solid yield 67 mg (63%): mp 193-208° with softening and swelling above 160°; [α]$_D^{21}$ +134 ± 3° (c 0.6, water); λ$_{KBr}^{max}$ 2.95-3.1 (OH, NH$_2$), 6.08, 6.25, 6.35, 6.8 (purine), 7.1, 7.52, 7.7, 8.1, 8.22, 9.2, 11.33, 12.55, 13.35, and 13.95 μm; λ$_{HClO}_4^{max}$ 210 (ε 18,900) and 262 nm (ε 14,500).

Anal. Calcd for C$_{11}$H$_{16}$N$_6$O$_5$: C, 44.59; H, 5.44; N, 28.37. Found: C, 44.39; H, 5.48; N, 28.04.

This compound was homogeneous by thin layer chromatography using methanol as developer.
25. 9-(2-Amino-2-deoxy-β-D-galactopyranosyl)adenine (XXIVc)

6-Benzamido-9-(3,4,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-β-D-galactopyranosyl)urine (XXIVA, 0.40 g) was deacylated with methanol nearly saturated at 0° with ammonia (80 ml) by the procedure described for the preparation of XVI. Attempted crystallization of the crude product from methanol-ether gave a white amorphous (234) solid, yield 0.155 g (61%); mp 212-220° with softening and swelling above 155°; $[\alpha]_D^{21} +19 \pm 1.5° (c \ 1.2, \text{ water}); \lambda_{\text{max}}^{\text{KBr}} 2.95-3.1 (\text{OH, NH}_2), 6.08, 6.25, 6.35, 6.6 (\text{p urine}), 7.08, 7.3, 7.53, 7.7, 8.0, 8.3, 9.22, 9.36, 11.3, 12.2, 12.55, and 13.75 μm; \lambda_{\text{max}}^{\text{H}_2\text{O}} 210 (c 19, 300) and 261 nm (c 14, 800).

Anal. Calcd for C_{11}H_{16}ClO_{5}: C, 44.59; H, 5.44; N, 28.37.

Found: C, 44.29; H, 5.58; N, 28.54.

This compound was homogeneous by thin layer chromatography using methanol as developer.

26. 9-(2-Amino-2-deoxy-α-D-galactopyranosyl)adenine Dihydrochloride (XXIIIId)

To a solution of 9-(2-amino-2-deoxy-α-D-galactopyranosyl adenine (XXIIIc, 40 mg) in methanol (30 ml) was added 0.40 ml of 1 N hydrochloric acid. Several evaporations with methanol (to a minimum volume of 3 ml) were made to remove excess hydrogen chloride. The solution was concentrated to 3-5 ml and warmed to 60°. Isopropyl ether (5 ml) was slowly added and the mixture allowed to cool slowly to room temperature then refrigerated overnight. The white crystalline material which formed was recrystallized from methanol-ethanol, yield 44 mg (88%); dec pt 208-209° with some charring above 190°; $[\alpha]_D^{22} +112 \pm 3° (c \ 0.6, \text{ water});$
\[ \lambda_{\text{max}}^{\text{KBr}} = 3.0-3.35 \text{ (OH, NH}_3^+), 5.78, 5.95, 6.18, 6.3, 6.5, 6.7, 6.85 \text{(purine)}, \\
7.05, 7.36, 7.46, 7.68, 8.2, 8.7, 8.85, 9.1, 9.4, 9.62, 9.83, 10.22, \\
11.43, 12.0, 12.5, \text{ and } 13.64 \mu \text{m}; \lambda_{\text{H}_2\text{O}}^{\text{max}} 210 \text{ (e } 17,800) \text{ and } 261 \text{ nm} \\
(e 14,200); \text{X-ray powder diffraction data } 13.92 \text{ s, 9.31 m, 8.41 vw,} \\
7.25 \text{ s (1), 6.63 w, 6.33 v, 4.43 s, 4.22 s, 3.89 s (3), 3.60 s, 3.36 m,} \\
3.20 \text{ s (2), 3.09 vw, 2.89 vw, 2.78 w, 2.69 w, 2.55 m, 2.37 m, 2.23 vw,} \\
2.14 v, \text{ and } 2.01 v. \]

**Anal.** Calcd for C_{11}H_{18}Cl_2N_6O_4: C, 35.73; H, 4.91; Cl, 19.21; \\
N, 22.76. Found: C, 35.70; H, 5.13; Cl, 19.27; N, 22.63.

27. **9-(2-Amino-2-deoxy-β-D-galactopyranosyl)adenine Dihydrochloride (XXIVd)**

A solution of 9-(2-amino-2-deoxy-β-D-galactopyranosyl)adenine (XXIVc, 0.10 g) in methanol (35 ml) was treated with 1 N hydrochloric acid (1.0 ml) by the procedure described in the preceding experiment. The yield after recrystallization from methanol-ethanol was 0.115 g (92%); dec pt 192-193° with some charring above 180°; [\alpha]_{D}^{22} +38 ± 2° (e 0.7, water); \[ \lambda_{\text{max}}^{\text{KBr}} = 2.95-3.4 \text{ (OH, NH}_3^+), 5.9, 6.0, 6.28, 6.66, \\
6.9 \text{(purine), 7.05, 7.54, 7.78, 8.14, 8.75, 8.9, 9.1, 9.45, 9.8,} \\
10.45, 10.66, 11.3, 11.8, 12.3, 12.8, 13.12, \text{ and } 13.8 \mu \text{m}; \lambda_{\text{H}_2\text{O}}^{\text{max}} 210 \\
(e 18,500) \text{ and } 260 \text{ nm} (e 14,000); \text{X-ray powder diffraction data } 6.89 \\
\text{vs (1), 6.35 v, 5.93 m, 5.52 w, 5.14 s, 4.81 m, 4.51 s, 4.28 m, 3.72 s,} \\
3.59 s, 3.43 s, 3.25 s (2), 3.13 s (3), 3.00 m, 2.93 w, 2.77 m, 2.67 \\
vw, 2.52 v, 2.43 m, 2.31 w, 2.26 vw, 2.13 v, 2.02 w, 1.90 vw, 1.84 w, \\
and 1.76 v. \]
II. Synthesis of 2-Amino-1,1,2-trideoxy-1-ethylthio-1-(1-thyminyl)-D-glucose Aldehyde

1. 2-Deoxy-2-trifluoroacetoamido-D-glucose Diethyl Dithioacetal (XXVI)

To a suspension of 2-amino-2-deoxy-D-glucose diethyl dithioacetal hydrochloride (224,225) (XXV, 3.0 g) in methanol (30 ml) was added an equivalent amount of sodium methoxide in methanol (0.22 g of sodium metal dissolved in 10 ml of methanol). The mixture was swirled until only a small residue (sodium chloride) remained. Ethyl trifluoro-thioacetate (2.0 g) was added and the resulting mixture kept at room temperature overnight. The solution was concentrated to a pale yellow residue which was extracted repeatedly with hot isopropyl ether (total 400 ml). The combined extracts were concentrated to 100 ml and refrigerated overnight. The white solid material which formed was recrystallized from isopropyl ether to give a white crystalline solid, yield 2.6 g (73%); mp 103-105°; [α]_D^23 -30 ± 1° (c 1.4, water); λ_{max}^{KBr} 3.0 (NH, OH), 5.85 (N-trifluoroacetyl carbonyl), 6.45 (NH), 8.5 (CF), 9.25, 9.52, 9.65, 9.85, 10.32, 10.7, 10.85, 11.2, 12.5, 12.92, 13.65, and 14.4 μm; X-ray powder diffraction data 13.10 s (2), 9.66 s, 8.49 s, 6.11 vw, 5.79 vw, 5.25 s (1), 5.04 m, 4.78 m, 4.46 s, 4.22 w, 4.10 m, 4.00 vw, 3.83 s, 3.58 v, 3.41 v, 3.30 m, 3.13 m, 2.99 m, 2.83 m, 2.68 vw, 2.57 vw, 2.48 vw, 2.41 v, 2.31 vw, 2.19 v, and 2.06 vw.
This compound was homogeneous by thin layer chromatography using acetone–chloroform (3:2) as developer.

2. 3,4,5,6-Tetra-O-acetyl-2-deoxy-2-trifluoroacetamido-D-glucose Diethyl Dithioacetal (XXVII)

2-Deoxy-2-trifluoroacetamido-D-glucose diethyl dithioacetal (XXVI, 1.2 g) was added to a pre-cooled (0°C) mixture of acetic anhydride (3.5 ml) and pyridine (8.0 ml) and the resulting mixture kept at room temperature overnight. The solution was poured into ice and water (20 ml) and the mixture extracted with dichloromethane (100 ml). The extract was washed with water and aqueous sodium bicarbonate then dried over anhydrous sodium sulfate. Evaporation of the solvent yielded a clear syrup which was crystallized from ethanol–water to give a colorless crystalline material, yield 1.6 g (93%): mp 66–67°C; [α]D220 +2 ± 0.5° (c 4.5, chloroform); λKBr max 3.05 (NH), 5.7–5.8 (O-acetyl and N-trifluoroacetyl carbonyl), 6.42 (NH), 8.15–8.35 (ester), 8.68 (CF), 6.98, 7.35, 8.5, 9.55, 9.77, 10.25, 10.65, 11.48, 11.65, 12.0, 12.6, 12.9, 13.3, 13.63, 13.85, and 14.25 μm; X-ray powder diffraction data 8.85 vs (1), 7.83 vs (3), 7.12 m, 6.61 s, 6.24 m, 5.57 s, 5.29 vs, 4.93 s, 4.71 s, 4.24 vs (2), 4.05 m, 3.92 vs, 3.78 m, 3.50 vs, 3.31 s, 3.20 vs, 3.07 m, 2.83 w, 2.77 m, 2.65 m, 2.56 m, 2.48 m, 2.41 vs, 2.35 w, 2.30 w, 2.26 w, 2.19 m, 2.03 m, 1.99 w, 1.96 w, 1.87 w, and 1.66 w.

Anal. Calcd for C12H22F3N05S2: C, 37.86; H, 5.81; N, 3.67; S, 16.8. Found: C, 38.06; H, 5.82; N, 3.78; S, 17.00.

Anal. Calcd for C20H30F3N09S2: C, 43.71; H, 5.50; N, 2.55; S, 11.67. Found: C, 43.53; H, 5.58; N, 2.58; S, 11.88.
3. 3,4,5,6-Tetra-O-acetyl-1,1,2-trideoxy-1-ethylthio-1-(thyminyl)-2-trifluoroacetamido-D-glucose Aldohydrol, (+) and (-) Forms (XXIX and XXXI)

3,4,5,6-Tetra-O-acetyl-2-deoxy-2-trifluoroacetamido-D-glucose diethyl dithioacetal (XXVII, 1.4 g) was dissolved in anhydrous ether (20 ml) and bromine (0.50 g) added. After standing at room temperature for 15 min, cyclohexene was added dropwise until no excess bromine remained (shown by change in color of the solution from bright red-orange to pale yellow). The solvent was then evaporated at 20° to yield a pale yellow syrup. Bis(trimethylsilyl)thymine (2.5 g) and chloroform (25 ml) were added and the mixture stirred until homogeneous. Evaporation of the solvent at 20° yielded a syrup which was heated at 120-130° for 15 min under diminished pressure (water aspirator). After cooling to room temperature, the product was added to 30% aqueous ethanol (50 ml) and the resulting mixture heated at 60° for 15 min. The solvent was evaporated and the resulting residue extracted with chloroform (120 ml). The chloroform extract was washed with water, dried (anhydrous sodium sulfate), and the solvent evaporated to yield a light brown syrup (1.28 g). Thin layer chromatography using ether as developer (2 developments) revealed three major components (Rf 0.95, 0.68, and 0.62). The crude product was resolved on 56 chromatoplates (200 X 200 X 1 mm) with ether as developer (each plate developed twice) and indication by uv light; the two slower moving zones (Rf 0.68 and 0.62) were removed and extracted with acetone. Evaporation of the extract from the faster of these two zones (Rf 0.68) gave a colorless glass. Attempted crystallization from ether-hexane gave 3,4,5,6-tetra-O-acetyl-1,1,2-
trideoxy-1-(thyminyl)-2-trifluoroacetamido-D-glucose aldehyde, (+) form (XXIX) as a white amorphous (234) solid, yield 0.24 g (15%):

mp 81-85°C with softening above 75°C; [a]$_D^{22}$ +53 ± 1° (c 1.2, chloroform);

λ$_{max}^{KBr}$ 3.1 (NH), 5.7 (α-acetyl and β-trifluoroacetyl carbonyl), 5.9, 6.45, 6.9 (NH, thymine), 8.2 (ester), 8.6 (CF), 7.3, 8.5, 9.0, 9.1, 10.52, 11.4, 11.75, 12.15, 12.9, 13.5, and 13.8 μm; λ$_{max}^{EtOH}$ 207 (ε 14,400) and 269 μm (ε 10,100).

Anal. Calcd for C$_{23}$H$_{31}$F$_3$N$_3$O$_{11}$S: C, 45.02; H, 4.93; N, 6.85; S, 5.23. Found: C, 45.02; H, 5.1; N, 6.57; S, 5.74.

This compound was homogeneous by thin layer chromatography using ether as developer (2 developments).

Evaporation of the extract from the slower zone (R$_f$ 0.62) gave a colorless glass which was crystallized from ether-hexane to give 3,4,5,6-tetra-α-acetyl-1,1,2-trideoxy-1-(thyminyl)-2-trifluoroacetamido-D-glucose aldehyde, (-) form (XXXI) as colorless crystals, yield 0.42 g (26%): mp 163-164°C; [a]$_D^{21}$ -90 ± 1° (c 1.2, chloroform);

λ$_{max}^{KBr}$ 3.1 (NH), 5.75 (α-acetyl and β-trifluoroacetyl carbonyl), 5.9, 6.46, 6.9 (NH, thymine), 8.1-8.3 (ester), 8.62 (CF), 8.5, 8.95, 9.22, 9.4, 9.52, 9.7, 10.26, 10.36, 10.55, 11.0, 11.4, 11.7, 11.9, 12.25, 13.0, 13.13, and 13.9 μm; λ$_{max}^{EtOH}$ 208 (ε 13,800) and 269 μm (ε 10,100); X-ray powder diffraction data 11.48 s, 9.99 w, 7.76 vs (1), 7.23 s, 5.91 w, 5.05 m, 4.76 vs (2), 4.52 w, 4.23 s, 4.08 w, 3.83 s (3), 3.60 s, 3.47 w, 3.33 m, 3.16 wv, 3.01 s, 2.91 w, 2.79 m, 2.63 m, 2.46 wv, 2.41 m, 2.22 w, 2.10 w, and 1.98 w.

Anal. Calcd for C$_{23}$H$_{31}$F$_3$N$_3$O$_{11}$S: C, 45.02; H, 4.93; N, 6.85; S, 5.23. Found: C, 44.97; H, 4.96; N, 7.09; S, 5.44.
This compound was homogeneous by thin layer chromatography using ether as developer (2 developments).

4. 2-Amino-1,1,2-trideoxy-1-ethylthio-
1-(thyminyl-D-glucose Aldehydrol Hydrochloride, (+) Form (XXXb)

3,4,5,6-Tetra-O-acetyl-1,1,2-trideoxy-1-ethylthio-1-(thyminyl)2-
trifluoroacetamido-D-glucose aldehydrol, (+) form (XXIX, 180 mg) was
dissolved in methanol saturated at 0° with ammonia. After standing
6 days at room temperature the solution was concentrated to a small
volume (5 ml) and ether (50 ml) added. The resulting flocculent pre-
cipitate was filtered to give 2-amino-1,1,2-trideoxy-1-ethylthio-1-
(thyminyl)-D-glucose aldehydrol (XXXa) as an amorphous solid which
quickly absorbed moisture from the air to become a brown gum. Attempted
crystallization of this compound was unsuccessful.

Crude XXXa was dissolved in methanol (25 ml) and 1 N hydrochloric
acid (25 ml) added. The solution was concentrated to a small volume
(5 ml) by evaporation at 20° and excess hydrogen chloride was removed
by repeated evaporation with methanol. The solution was evaporated to
a syrup which was dissolved in methanol (5 ml). Ether (30 ml) was
added and the resulting precipitate filtered. Attempted crystalliza-
tion from methanol-chloroform-ether gave a stable white amorphous (234)
solid, yield 89 mg (79%); mp 205-214°, with softening above 130° and
swelling above 150°; [α]_D^22 +93 ± 3° (c 0.5, water); λ\textsubscript{max}^KBr 3.0-3.35 (OH,
NH\textsubscript{3}⁺), 5.9, 6.7, 6.82 (thymine), 7.1, 7.3, 7.95, 8.2, 9.02, 9.3, 9.75,
10.25, 11.05, 11.3, and 12.9 μm; λ\textsubscript{max}^H₂O 208 (ε 8,920) and 270 nm (ε 8,360).

Anal. Calcd for C\textsubscript{13}H\textsubscript{24}ClN\textsubscript{3}O\textsubscript{6}S: C, 40.46; H, 6.27; Cl, 9.19; N,
10.89; S, 8.31. Found: C, 40.22; H, 6.39; Cl, 9.43; N, 10.44; S, 8.20.

5. 2-Amino-1,1,2-trideoxy-1-ethylthio-1-(thyminyl)-D-glucose Aldehydrol Hydrochloride, (−) Form (XXXIIb)

3,4,5,6-Tetra-O-acetyl-1,1,2-trideoxy-1-ethylthio-1-(thyminyl)-2-trifluoroacetamido-D-glucose aldehydrol, (−) form (XXXI, 0.25 g) was deacylated with methanolic ammonia and the crude product (XXXIIa) immediately converted to the hydrochloride (XXXIIb) by the procedure described in the preceding experiment. Attempted crystallization of the product from methanol–chloroform–ether gave a stable amorphous (234) solid, yield 0.12 g (76%); mp 199–211° with softening above 122° and swelling above 136°; [α]_D^{22} -107 ± 3° (ε 0.7, water); λ_{max}^{KBr} 3.0–3.42 (OH, NH_2^+), 5.9, 6.68, 6.82 (thymine), 7.3, 7.9, 8.22, 9.05, 9.3, 9.78, 10.25, 11.15, 12.5, and 13.25 μm; λ_{max}^{H_2O} 208 (ε 8,700) and 270 nm (ε 8,970).

Anal. Calcd for C_{13}H_{24}ClN_{10}O_{6}S: C, 40.46; H, 6.27; Cl, 9.19; N, 10.89, S, 8.31. Found: C, 40.37; H, 6.53; Cl, 9.46; N, 10.76; S, 8.05.

III. Synthesis of 1-(2-Amino-2-deoxy-β-D-glucofuranosyl)cytosine

1. Ethyl 2-Deoxy-1-thio-2-trifluoroacetamido-β-D-glucofuranoside (XXXIII)

(A) To a suspension of mercuric oxide freshly prepared from mercuric chloride (2.7 g) by the method of Pacsu and Wilson (226) in water (30 ml) was added 2-deoxy-2-trifluoroacetamido-D-glucose diethyl dithioacetal (XXVI, 2.8 g). Mercuric chloride (1.1 g) in water (50 ml) was added to the stirred suspension over a period of 2 hr. Stirring was continued for 30 min after the addition. The mixture was then
filtered through a pad of Celite and the filter cake washed with water (20 ml). The combined filtrate and washings were concentrated to a syrup which was dried by repeated evaporation with ethanol. Thin layer chromatography of the resulting syrupy residue using ethyl acetate-methanol (20:1) as developer revealed a major component ($R_f$ 0.64) and three minor components ($R_f$ 0.59, 0.25, and 0.10). The $R_f$ 0.59 component corresponded to starting material (XXVI). The crude product was heated in refluxing toluene for 1 hr. Evaporation of the solvent gave a solid residue (2.3 g). Thin layer chromatography of this residue revealed the absence of starting material. The crude product was finely powdered and mixed with Celite (2.0 g). The mixture was continuously extracted with benzene (300 ml) for 48 hr in a Soxhlet extraction apparatus. Evaporation of the benzene extract to dryness gave a solid residue which was recrystallized from acetone-hexane to give a white crystalline material, yield 0.37 g (37%), mp 143-146°. Further recrystallization from acetone-hexane afforded pure material; mp 149-151°; $[\alpha]_D^{21} +200 \pm 10$ (c 1.6, methanol); $\lambda_{\text{max}}^\text{KBr} 3.05$ (OH, NH), 5.88 (N-trifluoroacetyl carbonyl), 6.46 (NH), 8.55 (CF), 3.46, 6.8, 6.9, 7.3, 7.6, 7.7, 8.0, 8.44, 8.96, 9.2, 9.35, 9.65, 10.07, 10.36, 10.95, 11.2, 11.78, 12.68, 13.15, and 13.7 μm; X-ray powder diffraction data 14.73 m, 9.21 s, 7.56 vs (3), 6.97 s, 5.96 m, 5.10 vs (2), 4.76 s, 4.51 vs (1), 4.28 s, 4.04 m, 3.78 m, 3.48 vs (3), 3.37 wv, 3.19 m, 2.97 s, 2.82 w, 2.70 s, 2.57 m, 2.39 m, 2.33 m, 2.25 s, 2.17 m, 2.12 wv, 2.07 wv, 2.02 wv, and 1.96 w.

Anal. Calcd for C$_{10}$H$_{16}$F$_3$NO$_5$S: C, 37.62; H, 5.05; N, 4.39; S, 10.04. Found: C, 37.68; H, 5.37; N, 4.54; S, 10.51.
This compound was homogeneous by thin layer chromatography using acetone-chloroform (3:2) or ethyl acetate as developer.

(B) Ethyl 2-acetamido-3,5,6-tri-O-acetyl-2-deoxy-1-thio-α-D-glucofuranoside (227, 228) (XXXIV, 2.0 g), barium hydroxide octahydrate (15.0 g), and water (100 ml) were refluxed for 24 hr. The mixture was cooled and solid carbon dioxide was added until the the solution was neutral to phenolphthalein. After the addition of Celite (8 g), the mixture was filtered and the filtrate evaporated to dryness, the last traces of water being removed by repeated evaporation with absolute ethanol. The residue was extracted with absolute ethanol (120 ml) and the extract filtered to remove insoluble material. The solvent was evaporated from the filtrate to give a syrupy residue.

The syrupy residue (XXXV) was dissolved in absolute methanol (25 ml) and 2-ethyl trifluorothioacetate (1.0 g) was added. After 24 hr at room temperature, the solvent was evaporated and the residue was chromatographed through a silica gel (235) column (3.5 cm x 33 cm)

(235) Grade 950, 60-200 mesh; W. R. Grace, division of Davidson Chemical Co., Baltimore, Md.

with ethyl acetate as developer. After discarding the initial 50 ml, 1000 ml of eluent was collected and evaporated to a solid residue which was recrystallized from acetone-hexane, yield 1.15 g (70%), mp 145-148°. Recrystallization from acetone-hexane afforded pure material, mp and mmp with XXXIII prepared by the procedure described in part A 149-151°, [α]D21 + 201 ± 1° (c 1.3 methanol). X-ray powder diffraction data were identical to those for the compound prepared in part A.
2. Ethyl 3,5,6-Tri-O-acetyl-2-deoxy-1-thio-2-
trifluoroacetamido-α-D-glucofuranoside (XXXVI)

Ethyl 2-deoxy-1-thio-2-trifluoroacetamido-α-D-glucofuranoside
(XXXIII, 2.0 g) was added to a mixture of acetic anhydride (7 ml) and
pyridine (12 ml). After standing for 24 hr at room temperature, the
solution was poured, with stirring, into ice and water (30 ml) and the
mixture extracted with dichloromethane (100 ml). The dichloromethane
extract was washed with water and dried over anhydrous sodium sulfate.
Evaporation of the solvent from the extract gave a colorless syrup
which was crystallized from ether-hexane to give white crystalline
material, yield 2.55 g (91%): mp 72-74°; \([\alpha]_D^{21} +135 \pm 1° \text{ (c } 1.7,\text{ chloroform)}; \lambda_{max}^{KBr} 3.05 \text{ (NH), 5.72 (O-acetyl carbonyl), 5.85 (N-}
trifluoroacetyl carbonyl), 6.46 \text{ (NH), 8.0-8.3 (ester), 8.6 (CF), 3.3,}
3.45, 6.92, 7.3, 8.4, 9.1, 9.42, 9.63, 10.23, 10.55, 10.75, 11.05, 11.28,
11.46, 11.77, and 13.5 μm; X-ray powder diffraction data 10.05 vs (1),
8.27 w, 6.81 w, 4.96 vs (2), 4.76 m, 4.59 s, 4.38 s, 4.11 s (3), 3.96
w, 3.88 s, 3.74 w, 3.61 s, 3.48 w, 3.36 w, 3.25 m, 3.16 m, 2.82 w,
and 2.75 w.

**Anal. Calcd for C₁₆H₂₂F₃NO₅S: C, 43.14; H, 4.98; N, 3.14; S,**
7.20. **Found: C, 43.00; H, 5.36; N, 3.36; S, 7.69.**

This compound was homogeneous by thin layer chromatography using
ether-hexane (2:1) as developer.
3. 1-(3,5,6-Tri-O-acetyl-2-deoxy-2-
trifluoroacetamido-β-D-glucosyl)furanosyl)cytosine (XXXVIII)

Ethyl 3,5,6-tri-O-acetyl-2-deoxy-1-thio-2-trifluoroacetamido-α-D-
glucoside (XXXVI, 2.0 g) was dissolved in dichloromethane (20 ml) and dry chlorine was passed through the solution for 15 min. Evaporation of the solvent gave a pale yellow syrup which was redissolved in dichloromethane (10 ml) and a few drops of cyclohexene added. After evaporation of the solvent, the residual syrup was dissolved in chloroform (10 ml), bis(trimethylsilyl)cytosine added, and the mixture stirred until homogeneous. The solvent was evaporated and the residue heated at 130-140° for 12 min under diminished pressure (water aspirator). After cooling, the mixture was added to 80% ethanol containing sodium bicarbonate (0.6 g) and the mixture heated at 60° for 15 min. The solvent was then evaporated and the residue extracted with chloroform (200 ml). The dried (sodium sulfate) extract was evaporated to dryness and the residue (1.4 g) crystallized slowly from methanol-isopropyl ether, yield 0.27 g (12%); mp 257-259°. Recrystallization from methanol-isopropyl ether afforded pure material: mp 262-264°; [α]D +25
± 1° (c 1.1, methanol); λMAXKBr 3.1 (NH2), 5.7-5.8 (O-acetyl and N-tri-
fluoroacetyl carbonyl), 6.05-6.15, 6.4, 6.72 (NH, cytosine), 8.1-8.3
(ester), 8.62 (CF), 7.32, 7.8, 8.62, 9.5, 10.5, 10.95, 11.75, 12.0,
12.5, and 14.3 μm; λMAXKOH 206 (ε 20,000), 244 (shoulder, ε 8,870), and
270 nm (ε 9,020); X-ray powder diffraction data 10.05 m, 8.51 vs (1),
7.50 m, 6.86 vw, 6.37 s, 5.87 vs, 5.54 vw, 5.23 m, 4.93 vs (3), 4.58 s,
4.36 vs (2), 4.10 s, 3.94 s, 3.73 vs (3), 3.55 m, 3.42 w, 3.31 w,
3.19 m, 3.11 w, 2.88 s, 2.75 m, 2.62 w, 2.45 w, 2.33 w, 2.27 w, 2.22 w, 2.12 m, 1.94 w, 1.88 m, and 1.78 w.

**Anal.** Caled for C_{16}H_{21}F_{3}N_{4}O_{9}: C, 43.73; H, 4.29; N, 11.33.

Found: C, 43.61; H, 4.40; N, 11.59.

This compound was homogeneous by thin layer chromatography using ethyl acetate-methanol (10:1) or acetone-chloroform (3:1) as developer. Thin layer chromatography of the mother liquors using acetone-chloroform (3:1) as developer revealed two major overlapping components (R_f 0.8 and 0.7) and three minor components (R_f 0.45, 0.25 and 0.10). The R_f 0.25 component corresponded to XXXVIII. These five components were isolated by preparative thin layer chromatography using acetone-chloroform (5:2) as developer. Crystallization of the R_f 0.25 component from methanol-isopropyl ether gave an additional 0.05 g (2.3%) of XXXVIII. The other four components were examined by infrared and ultraviolet spectroscopy. None of these components showed absorptions characteristic of cytosine nucleoside derivatives.

4. 1-(2-Amino-2-deoxy-β-D-glucofuranosyl)cytosine sulfate (XXXIXb)

1-(3,5,6-Tri-O-acetyl-2-deoxy-2-trifluoroacetamido-β-D-glucofuranosyl)cytosine (XXXVIII, 0.30 g) was dissolved in methanol saturated at 0° with ammonia (50 ml). After standing 7 days at room temperature, the solution was concentrated to a small volume (5 ml) and ether (60 ml) was added. The resulting flocculent precipitate was filtered and washed with ether (20 ml) to give 1-(2-amino-2-deoxy-β-D-glucofuranosyl)-cytosine (XXXIXa) as a white amorphous solid.
Crude XXXIXa was dissolved in methanol (10 ml) and 6 N sulfuric acid (0.15 ml) added. The precipitate which formed was filtered, washed with ether and crystallized from methanol, yield, 0.19 g (85%); mp 238-240° dec with charring above 130°; [α]D21 +42 ± 2° (c 0.4, water); optical rotatory dispersion spectrum (figure 16) [M]20 282 +9,000 ± 800° (pk), [M]20 238 -13,500 ± 800° (tr) (c 0.01, water); λmax KBr 3.0-3.1 (SH), 3.25-3.4 (NH3+), 5.8, 6.05, 6.25, 6.52 (cytosine), 7.06, 7.48, 7.58, 8.05, 8.6, 9.0-9.2, 9.62, 10.3, 11.25, 11.95, 12.8, and 13.15 μm; λmax H2O 202 (ε 15,700), 230 (shoulder, ε 7,440), and 271 nm (ε 8,700); λmax 0.1 N HCl 212 (ε 10,700) and 278 nm (ε 13,900); X-ray powder diffraction data 10.34 vs (2), 7.79 s, 6.76 vw, 6.37 m, 5.63 s, 5.14 s, 4.78 s, 4.45 vs (1), 4.13 s, 3.88 s, 3.76 m, 3.48 vs (3), 3.36 m, 3.25 m, 3.16 w, 3.10 v, 3.03 m, 2.92 m, 2.83 s, 2.75 w, 2.71 w, 2.64 m, 2.57 s, 2.43 m, 2.36 m, 2.29 w, 2.23 s, 2.15 m, 2.11 vw, 2.06 w, 2.00 w, and 1.96 s.

Anal. Calcd for C₁₀H₁₈N₄O₉S: C, 32.43; H, 4.90; N, 15.13; S, 8.66.
Found: C, 32.32; H, 5.20; N, 15.23; S, 8.70.

- 5. Attempted Condensation of 3,5,6-Tri-O-acetyl-2-deoxy-2-(2,4-dinitroanilino)-D-glucosyl chloride with Bis(trimethylsilyl)cytosine

Ethyl 3,5,6-tri-O-acetyl-2-deoxy-2(2,4-dinitroanilino)-1-thio-α-D-glucosyl, prepared by the procedure of Wolfrom and Winkley (228), (1.0 g) was dissolved in dichloromethane (20 ml) and dry chlorine passed through the solution for 15 min. Evaporation of the solvent gave a yellow syrup which was redissolved in dichloromethane (10 ml) and a few drops of cyclohexene added. After evaporation of the solvent, the
residual syrup was dissolved in chloroform (10 ml) and bis(trimethylsilyl)cytosine (1.5 g) added. After stirring until homogeneous, the solvent was evaporated and the residue heated at 130-140° for 15 min under diminished pressure (water aspirator). After cooling to room temperature, the mixture was added to 80% ethanol (40 ml) containing sodium bicarbonate (0.3 g) and the mixture heated at 60° for 15 min. The solvent was evaporated and the residue extracted with chloroform (100 ml). The dried (sodium sulfate) extract was evaporated to dryness to give a yellow glass (0.9 g). Attempted crystallization of this crude product was unsuccessful. Thin layer chromatography of the crude product using ethyl acetate-methanol (10:1) as developer revealed six major components (Rf 0.80, 0.66, 0.60, 0.38, 0.30, and 0.12). These components were isolated by preparative thin layer chromatography and examined by ultraviolet and infrared spectroscopy. None showed absorptions characteristic of cytosine nucleoside derivatives. In particular, the absence of a strong absorption in the 6.0-6.1 μm range was noted. Several minor components of the crude product were also noted by thin layer chromatography but were not isolated.

IV. Synthesis of 1-(2-Amino-2-deoxy-β-D-xylofuranosyl)thymine

1. Ethyl 2-Deoxy-1-thio-2-
   trifluoroacetamido-α-D-xylofuranoside (XL)

To a cold (10°) solution of ethyl 2-deoxy-1-thio-2-trifluoroacetamido-α-D-glucofuranoside (XXXIII, 1.91 g) in 50% methanol (40 ml) was added a solution of sodium metaperiodiate (1.35 g, 1:1.05 molar ratio) in water (25 ml) at 10°. The reaction was left in the dark at 10° for
30 min. A solution of barium chloride dihydrate (0.77 g) in water (6 ml) was added and the resulting precipitate of barium iodate was removed by filtration.

To the resultant stirred solution was added dropwise a solution of sodium borohydride (0.30 g) in water (6 ml) over a period of 10 min. After stirring for an additional 30 min the solution was neutralized to pH 7 with 1 N sulfuric acid. The solution was concentrated to a small volume and the remaining water removed by repeated evaporation with absolute ethanol. The resulting solid residue was extracted with acetone (100 ml). The solvent was evaporated from the extract and the residue was chromatographed through a silica gel (235) column (3.3 X 35 cm) with ethyl acetate as developer. After discarding the first 50 ml, 1000 ml of eluent was collected and the solvent evaporated. The residue was recrystallized from acetone-hexane, yield 1.26 g (73%), mp 135-138°. Recrystallization from acetone-hexane afforded pure material: mp 139-141°; \([\alpha]^{20}_D\) +242 ± 1° (c 2.5, methanol); \(\lambda_{\text{max}}^{\text{KBr}}\) 3.0-3.1 (OH, NH), 5.88 (N-trifluoroacetyl carbonyl), 6.45 (NH), 8.62 (CF), 3.42, 6.8, 7.3, 7.5, 7.68, 7.96, 8.2-8.4, 9.1, 9.56, 9.7, 9.82, 10.85, 11.52, 12.1, 12.75, 13.08, and 13.6 µm;

X-ray powder diffraction data 7.44 s (2), 5.01 s (3), 4.31 vs (1), 4.10 m, 4.03 m, 3.90 w, 3.71 m, 3.26 w, 3.11 w, 2.95 s, 2.81 m, 2.69 w, 2.55 w, 2.42 w, 2.34 w, 2.28 w, 2.22 w, 2.12 w, 2.06 w, and 1.97 w.

Anal. Calcd for C_{9}H_{14}F_{3}NO_{4}S: C, 37.37; H, 4.88; N, 4.84; S, 11.08. Found: C, 37.64; H, 5.17; N, 5.16; S, 11.53.

This compound was homogeneous by thin layer chromatography using chloroform-acetone (1:1) or ethyl acetate as developer.
2. Ethyl 3,5-Di-O-acetyl-2-deoxy-1-thio-2-trifluoroacetamido-α-D-xylofuranoside (XLI)

Ethyl 2-deoxy-1-thio-2-trifluoroacetamido-α-D-glucofuranoside (XL, 2.20 g) was added to a mixture of acetic anhydride (6 ml) and pyridine (10 ml). After standing for 24 hr at room temperature, the solution was oured, with stirring, into ice and water (30 ml) and the resulting mixture extracted with dichloromethane (100 ml). The dichloromethane extract was washed with water and dried over anhydrous sodium sulfate. Evaporation of the solvent gave a colorless syrup which was crystallized from ether-hexane, yield 2.38 g (84%); mp 60-61.5°, [α] \(_D^{21} \) +159 ± 1° (c 3.3, chloroform); λ\(_{\text{max}}^{\text{KBr}} \) 3.1 (NH), 5.75 (O-acetyl carbonyl), 5.85 (N-trifluoroacetyl carbonyl), 6.42 (NH), 8.0-8.2 (ester), 8.6 (CF), 7.28, 8.28, 8.43, 9.03, 9.55, 9.8, 10.6, 11.36, 12.05, and 13.5 µm; X-ray powder diffraction data 9.41 s (1), 6.37 w, 5.44 w, 4.89 s (3), 4.58 s (2), 4.33 m, 4.10 s (3), 3.93 m, 3.74 m, 3.58 w, 3.45 w, 3.24 m, 3.04 m, 2.91 vw, 2.84 vw, 2.71 w, 2.64 w, 2.53 m, 2.34 w, and 2.24 w.

Anal. Calcd for C\(_{13}\)H\(_{18}\)F\(_3\)NO\(_5\)S: C, 41.82; H, 4.86; N, 3.75; S, 8.59. Found: C, 41.86; H, 4.83; N, 4.03; S, 9.04.

This compound was homogeneous by thin layer chromatography using ether-hexane (2:1) as developer.
3. 1-(3,5-Di-2-acetyl-2-deoxy-2-
trifluoroacetamido-β-D-xylofuranosyl)thymine (XLIII)

Ethyl 3,5-di-2-acetyl-2-deoxy-1-thio-2-trifluoroacetamido-α-D-
xylofuranoside (XLI, 1.35 g) was dissolved in dichloromethane (20 ml)
and dry chlorine was passed through the solution for 15 min. Evapora-
tion of the solvent gave a pale yellow syrup which was redissolved in
dichloromethane (10 ml) and a few drops of cyclohexene added. After
evaporation of the solvent, the residual syrup was dissolved in
chloroform (10 ml). Bis(trimethylsilyl)thymine (119, 123) (2.0 g)
was added and the mixture stirred until homogeneous. The solvent was
evaporated and the residue was heated at 110-120° for 15 min under
diminished pressure (water aspirator). After cooling to room tempera-
ture, 80% ethanol (30 ml) was added and the mixture heated at 60°, with
stirring, for 15 min. The solvent was evaporated and the residue
extracted with chloroform (200 ml). The chloroform extract was washed
with water, dried over anhydrous sodium sulfate and evaporated to dry-
ness to yield a pale amber glass (1.6 g). Thin layer chromatography
of the product using chloroform-acetone (3:2) as developer revealed two
major components (R_f 0.85 and 0.55). The crude product was resolved on
24 chromatoplates (200 X 200 X 1 mm) with chloroform-acetone (3:2) as
developer and indication by uv light. The two major zones were removed
and extracted with acetone. Evaporation of the extract from the slower
(R_f 0.55) zone gave a clear glass (1.05 g). Attempted crystallization
from chloroform-isopropyl ether gave 1-(3,5-di-2-acetyl-2-deoxy-2-
trifluoroacetamido-β-D-xylofuranosyl)thymine (XLIII) as a white amor-
phous (234) solid, yield 0.96 g (61%): mp 138-145° with softening
above 85°; [α]_{D}^{21} +41 ± 1° (c 3.1, chloroform); \lambda_{\text{max}}^{\text{KBr}} 3.1 (\text{NH}), 5.7-5.8 (N-acetyl and N-trifluoroacetethyl carbonyl), 5.9, 6.42, 6.8 (NH, thymine), 8.05-8.2 (ester), 8.6 (CF), 7.3, 8.45, 8.96, 9.5, 11.08, and 12.7 µm; 
\lambda_{\text{max}}^{\text{EtOH}} 208 (ε 12,000) and 265 nm (ε 9,650).

Anal. Calcd for C_{16}H_{18}F_{3}N_{3}O_{6}: C, 43.94; H, 4.15; N, 9.61. Found: C, 44.07; H, 4.51; N, 9.40.

This compound was homogeneous by thin layer chromatography using chloroform-acetone (3:2) or ethyl acetate-benzene (5:2) as developer.

Evaporation of the extract from the faster (Rf 0.85) zone gave a clear glass (0.28 g). The infrared spectrum of this substance closely resembled that of the starting material (XLII). This spectrum lacked absorptions in the 5.9-6.2 and 6.6-6.9 m ranges characteristic of thymine nucleoside derivatives. The ultraviolet spectrum of this substance also showed no absorption bands above 210 nm. It was concluded, therefore, that this substance was not a nucleoside and it was not further investigated. Several other minor components were observed but were not isolated. It was estimated that none of these was present in more than 5-8% yield.

4. 1-(2-Amino-2-deoxy-ß-D-xylofuranosyl)thymine Hydrochloride (XLIVb)

1-(3,5-Di-N-acetyl-2-deoxy-2-trifluoroacetamido-ß-D-xylofuranosyl)-thymine (XLIII, 0.40 g) was dissolved in methanol saturated at 0° with ammonia. After standing for 7 days at room temperature, the solution was concentrated to a small volume (5 ml) and ether was added (60 ml). The resulting flocculent precipitate was collected by filtration and washed with ether to give 1-(2-amino-2-deoxy-ß-D-xylofuranosyl)thymine
(XLIVa) as a white amorphous solid. Attempted crystallization from methanol–ether was unsuccessful.

Crude XLIVa was dissolved in methanol (20 ml) and 2 N hydrochloric (0.8 ml) was added. The solvent was evaporated and excess hydrogen chloride was removed by repeated evaporation with ethanol. The residue was dissolved in methanol (15 ml) and ether (50 ml) was added. The resulting precipitate was filtered, washed with ether, and crystallized from methanol–ether to give a white crystalline material, yield 0.23 g (86%); mp 226–227° dec; [α]21\textsuperscript{D} +18 ± 1° (c 1.2, water); optical rotatory dispersion spectrum (figure 18) [M]\textsuperscript{20} 283 +4,400 ± 500° (pk) and [M]\textsuperscript{252} -8,000 ± 600° (tr) (c 0.09, water); λ\textsuperscript{H2O}\textsuperscript{max} 2.9–3.0 (OH), 3.15–3.4 (NH\textsuperscript{3+}), 5.85, 6.65, 6.83 (thymine), 7.12, 7.28, 7.78, 8.32, 8.7, 9.10, 9.5, 9.82, 10.2, 10.8, 12.3, 12.7, 12.9, and 13.7 μm; λ\textsuperscript{H2O}\textsuperscript{max} 207 (ε 9,080) and 267 nm (ε 9,290); X-ray powder diffraction data 10.22 vw, 8.27 vs (1), 6.13 m, 5.79 m, 5.43 m, 4.87 m, 4.53 s, 4.19 s (3), 3.90 s, 3.69 s, 3.48 s, 3.27 s (2), 3.16 s (2), 3.04 vw, 2.93 m, 2.84 m, 2.65 m, 2.54 m, 2.51 w, 2.45 w, 2.39 w, 2.32 m, 2.22-w, 2.12 w, 1.99 w, and 1.87 w.

Anal. Calcd for C\textsubscript{10}H\textsubscript{16}ClN\textsubscript{3}O\textsubscript{5}: C, 40.88; H, 5.49; Cl, 12.07; N, 14.30. Found: C, 41.07; H, 5.30; Cl, 11.79; N, 14.32.
SUMMARY

1. Condensation of 3,4,6-tri-O-acetyl-2-deoxy-a-D-glucopyranosyl bromide with bis(trimethylsilyl)cytosine yielded 1-(3,4,6-tri-O-acetyl-2-deoxy-a-D-glucopyranosyl)cytosine. Treatment of this compound with ammoniacal benzyl alcohol followed by catalytic hydrogenation under mild conditions gave 1-(2-amino-2-deoxy-β-D-glucopyranosyl)cytosine.

2. Treatment of 2-amino-2-deoxy-D-glucose with S-ethyl trifluoro-thioacetate followed by acetylation with acetic anhydride-pyridine gave 1,3,4,6-tetra-O-acetyl-2-deoxy-2-trifluoroacetamido-a-D-glucopyranose and the corresponding β-D anomer. Treatment of these compounds with hydrogen bromide in acetic acid yielded 3,4,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-a-D-glucopyranosyl bromide.


4. Condensation of 3,4,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-a-D-glucopyranosyl bromide with 6-benzamido-9-chloromercuripurine gave the anomeric 6-benzamido-9-(3,4,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-β-D-glucopyranosyl)purines. Deblocking of these compounds with
methanolic ammonia yielded 9-(2-amino-2-deoxy-α-D-glucopyranosyl)adenine and the corresponding β-D anomer.

5. Treatment of 2-amino-2-deoxy-α-D-galactose with S-ethyl trifluorothioacetate followed by acetylation with acetic anhydride-pyridine gave 1,3,4,6-tetra-O-acetyl-2-deoxy-2-trifluoroacetamido-α-D-glucopyranose and the corresponding β-D anomer. Treatment of these compounds with hydrogen bromide in acetic acid yielded 3,4,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-α-D-galactopyranosyl bromide.


9. Treatment of 2-amino-2-deoxy-D-glucose diethyl dithioacetal with S-ethyl trifluorothioacetate gave 2-deoxy-2-trifluoroacetamido-
D-glucose diethyl dithioacetals. Acetylation of this compound with acetic anhydride-pyridine gave 3,4,5,6-tetra-O-acetyl-2-deoxy-2-trifluoroacetamido-D-glucose diethyl dithioacetals.

10. Treatment of 3,4,5,6-tetra-O-acetyl-2-deoxy-2-trifluoroacetamido-D-glucose diethyl dithioacetals with a molar equivalent of bromine yielded 3,4,5,6-tetra-O-acetyl-1-bromo-1,1,2-trideoxy-1-ethylthio-2-trifluoroacetamido-D-glucose aldehyde, which was immediately condensed with bis(trimethylsilyl)thymine to give the two 1-epimers of 3,4,5,6-tetra-O-acetyl-1,1,2-trideoxy-1-ethylthio-1-(1-thyminyl)-2-trifluoroacetamido-D-glucose aldehyde. Depolymerization of these two compounds with methanolic ammonia gave the two 1-epimers of 2-amino-1,1,2-trideoxy-1-ethylthio-1-(1-thyminyl)-D-glucose aldehyde isolated as their hydrochlorides.


12. Reaction of ethyl 3,5,6-tri-O-acetyl-2-deoxy-1-thio-2-trifluoroacetamido-a-D-glucofuranoside with dry chlorine yielded 3,5,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-D-glucofuranosyl chloride which was immediately condensed with bis(trimethylsilyl)cytosine to
give 1-(3,4,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-β-D-glucofuranosyl)cytosine. Decacylation of this compound with methanolic ammonia gave 1-(2-amino-2-deoxy-β-D-glucofuranosyl)cytosine isolated as the sulfate.


14. Reaction of 3,5-di-O-acetyl-2-deoxy-1-thio-2-trifluoroacetamido-α-D-xylofuranoside with dry chlorine yielded 3,5-di-O-acetyl-2-deoxy-2-trifluoroacetamido-D-xylofuranosyl chloride which was immediately condensed with bis(trimethylsilyl)thymine to give 1-(2,5-di-O-acetyl-2-deoxy-2-trifluoroacetamido-β-D-xylofuranosyl)thymine. Decacylation of this compound with methanolic ammonia gave 1-(2-amino-2-deoxy-β-D-xylofuranosyl)thymine isolated as the hydrochloride.
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