N9 Alkylation and Glycosylation of Purines; A Practical Synthesis of 2-Chloro-2'-deoxyadenosine

Minghong Zhong
Brigham Young University - Provo
N9 ALKYLATION AND GLYCOSYLATION OF PURINES; A PRACTICAL
SYNTHESIS OF 2-CHLORO-2’-DEOXYADENOSINE

by

Minghong Zhong

A dissertation submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of Chemistry and Biochemistry
Brigham Young University
August 2004
BRIGHAM YOUNG UNIVERSITY

GRADUATE COMMITTEE APPROVAL

of a dissertation submitted by

Minghong Zhong

This dissertation has been read by each member of the following graduate committee and by majority vote has been found to be satisfactory.

05/19/04
Date

Morris J. Robins, Chair

5/19/04
Date

Gerald D. Watt

5/19/04
Date

Merritt B. Andrus

5/19/04
Date

Paul B. Savage

5/19/04
Date

Matt A. Peterson
As chair of the candidate’s graduate committee, I have read the dissertation of Minghong Zhong in its final form and have found that (1) its format, citations, and bibliographical style are consistent and acceptable and fulfill university and department style requirements; (2) its illustrative materials including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory to the graduate committee and is ready for submission to the university library.

05/19/04
Date

Morris J. Robins
Chair, Graduate Committee

Accepted for the Department

Noel L. Owen
Graduate Coordinator

Accepted for the College

G. Rex Bryce, Associate Dean
College of Physical and Mathematical Sciences
ABSTRACT

N9 ALKYLATION AND GLYCOSYLATION OF PURINES; A PRACTICAL SYNTHESIS OF 2-CHLORO-2’-DEOXYADENOSINE

Minghong Zhong

Department of Chemistry and Biochemistry

Doctor of Philosophy

(a) The Robins reagent [2-acetamido-6-O-(diphenylcarbamoyl)purine] was utilized for glycosylation under Lewis acid conditions. Regioselectivity of glycosylation depends on the glycosyl donor and its 2-O- or 2-N-protecting group. Regioselective N9 glycosylation of 2-acetamido-6-O-(diphenylcarbamoyl)purine with problematic glucosamine has been accomplished by protecting the amino function as a phthalimido group with consequent stabilization of the oxocarbenium cation, and lowering the activation energy by introduction of trichloroacetimidate at the anomeric carbon.

(b) 6-Heteroaryl functions [6-(1,2,4-triazol-4-yl) and 6-(imidazol-1-yl)] were introduced into purine derivatives for regioselective N9 alkylation. The regiospecificity of alkylation mainly results from steric effects due to the coplanar conformation of the two linked heterocyclic rings governed by conjugation. Several of the obtained acyclic derivatives showed antiviral and antitumor activities.
(c) Glycosylation of purine derivatives with 2-deoxy-3,5-di-O-(p-toluoyl)-α-D-erythro-pentofuranosyl chloride using the sodium salt method usually gave a mixture of both anomers. Lipophilic groups were introduced into the imidazole ring of 6-(imidazol-1-yl)purine derivatives to increase the solubility of the sodium salts in moderately polar solvents. Differential solvation effects in binary solvent mixtures were utilized to improve the stereoselectivity of glycosylation. The stereoselectivity varied with the sizes of lipophilic groups and the polarity of solvents. With the propyl group, and in CH₃CN/toluene (1:1) and/or CH₃CN/CH₂Cl₂ (1:1), regiospecific and highly stereoselective glycosylation of purines with 2-deoxy-3,5-di-O-(p-toluoyl)-α-D-erythro-pentofuranosyl chloride was achieved.

(d) Using the above method, a low cost and efficient synthesis of 2-chloro-2’-deoxyadenosine (2-CdA, cladribine) was accomplished with an overall yield of 48% from inexpensive guanosine and 57% from 2,6-dichloropurine. 2-Chloro-6-(2-propylimidazol-1-yl)purine was prepared either from guanosine in a yield of 61% in 5 steps or from 2,6-dichloropurine in a yield of 72% in one step. Coupling of this 2-chloro-6-heteroarylpurine with 2-deoxy-3,5-di-O-(p-toluoyl)-α-D-erythro-pentofuranosyl chloride in binary solvent mixtures, followed by activation of imidazolyl as a better leaving group via benzylation at N3 and then ammonolysis gave cladribine in good yield (79%) for 3 steps. Analogos of purine derivatives with lipophilic groups (butyl, pentyl and 2-phenylpropyl) worked almost as well.
ACKNOWLEDGMENTS

I would like to thank my wife Xiaoyun and my son Chiyuan for their love and support. They have shown me their great patience and enduring spirit in these past years. I thank my parents for their love, commendation of hard work and their encouragement of being a better person.

I would like to give my special thanks to my mentor, Dr. Robins, for his continuous guidance and support of my research, his patience with me and his confidence in my abilities.

I would also like to thank the professors who taught me chemistry, and shared with me their enthusiasm for chemical research. I am grateful to my advisory committee and other members of the department for their instruction, help and friendship.

I thank Dr. John F. Cannon, Mr. Bruce Jackson and Dr. Du Li for their assistance in the structure determination of compounds, and thank Dr. Matthew C. Asplund and Dr. Randall B. Shirts for their help with computation of molecular properties.

I am grateful for the financial support of this work by Brigham Young University Department of Chemistry and Biochemistry, Brigham Young University Fellowship, Roland K. Robins Fellowship and the Brigham Young University Cancer Center Fellowship.
TABLE OF CONTENTS

LIST OF ABBREVIATIONS.................................................................xii
GENERAL EXPERIMENTAL PROCEDURES..........................................xiv
LIST OF TABLES.............................................................................xvi
LIST OF FIGURES...........................................................................xviii
LIST OF SCHEMES.........................................................................xxi

CHAPTER 1. INTRODUCTION: METHODS OF NUCLEOSIDE SYNTHESIS

1. General Methods of Nucleoside Synthesis by Direct Glycosylation ..............1
   1.1. Hilbert-Johnson Method.................................................................1
   1.2. Fischer-Helferich Silver Salt Method and Mercury Salt Method...............2
   1.3. Fusion Method.............................................................................3
   1.4. Transglycosylation Method.............................................................4
   1.5. Enzymatic Synthesis.................................................................5
   1.6. Vorbrüggen Glycosylation (Silyl-Hilbert-Johnson Method).......................6
   1.7. Sodium Salt Method................................................................7

2. Strategies for Stereoselective Glycosylation of 2-Deoxy-α-erythro-pentofuranose Derivatives.................................................................9
   2.1. Anchimeric Assistance of 2-α-Substituents.........................................10
   2.2. Anchimeric Assistance of 3-α-Substituents.........................................13
   2.3. 5’-Tethered Bases for Intramolecular Glycosylation.............................16
   2.4. Miscellaneous Methods.............................................................19

3. Strategies for Regioselective Alkylolation and Glycosylation of Purines...........20
CHAPTER 2. GLYCOSYLATION OF 2-N-ACETYL-6-O-(DIPHENYLCARBAMOYL)GUANINE WITH RIBOFURANOSE AND GLUCOPYRANOSE DERIVATIVES WITH LEWIS ACID CATALYSTS

1. Introduction

2. Results and Discussion

2.1. Glycosylation of 2-N-Acetyl-6-O-(diphenylcarbamoyl)guanine with 1-O-Acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose

2.2. Glycosylation of 2-N-Acetyl-6-O-(diphenylcarbamoyl)guanine with 1,2,3,4,6-Penta-O-acetyl-α-D-glucopyranose

2.3. Glycosylation of 2-N-Acetyl-6-O-(diphenylcarbamoyl)guanine with 2-Acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-α-D-glucopyranose

2.4. Glycosylation of 2-N-Acetyl-6-O-(diphenylcarbamoyl)guanine with 1,3,4,6-Tetra-O-acetyl-2-deoxy-2-phthalimido-D-glucopyranose

2.5. Glycosylation of 2-N-Acetyl-6-O-(diphenylcarbamoyl)guanine with 3,4,6-Tri-O-acetyl-1-O-trichloroacetamidoyl-2-deoxy-2-phthalimido-β-D-glucopyranose

3. Conclusions

4. Experimental Section
CHAPTER 3. SYNTHESSES OF SELECTED 6-HETEROARYLPURINES AND THEIR REGIOSPECIFIC ALKYULATION TO GIVE N9 ALKYL DERIVATIVES

1. Introduction

2. Results and Discussion
   2.1. Syntheses of Selected 6-Heteroarylpurines
      2.1.1. 6-(1,2,4-Triazol-4-yl)purine
      2.1.2. 6-(Imidazol-1-yl)purine
      2.1.3. 2-Chloro-6-(imidazol-1-yl)purine
      2.1.4. 2-Amino-6-(imidazol-1-yl)purine and 2-Acetamido-6-(imidazol-1-yl)purine

2.2. Regiospecific 9-Alkylation
   2.2.1. Alkylation of 6-(1,2,4-Triazol-4-yl)purine
   2.2.2. Alkylation of 6-(Imidazol-1-yl)purine
   2.2.3. Alkylation of 2-Amino-6-(imidazol-1-yl)purine
   2.2.4. Alkylation of 2-Chloro-6-(imidazol-1-yl)purine

2.3. Steric and Electronic Effects of the 6-Heteroaryl groups on Alkylations of Purines

2.4. Biological Assays

3. Conclusions

4. Experimental Section

5. References and Notes
CHAPTER 4. GLYCOSYLATION OF 6-HETEROARYLPURINES,  
REGIOSELECTIVITY AND STEREoseLECTIVITY

1. Introduction .............................................................................................................. 144

2. Results and Discussion
   2.1. Attempts at Glycosylation of Ribofuranose Derivatives Using 6-(Imidazol-1-yl)purines ......................................................................................................................... 149
      2.1.1. Glycosylation of Ribofuranose Derivatives ....................................................... 149
   2.2. Glycosylation of 2-Deoxy-D-erythro-pentofuranose Derivatives by the Sodium Salt Procedures
      2.2.1. Quantitative $^1$H NMR .................................................................................. 152
      2.2.2. Chemistry of 2-Deoxy-3,5-di-O-($p$-toluoyl)-$\alpha$-D-erythro-pentofuranosyl Chloride ......................................................................................................................... 157
      2.2.3. Coupling of 6-(Imidazol-1-yl)- and 6-(1,2,4-Triazol-4-yl)purine with 2-Deoxy-3,5-di-O-($p$-toluoyl)-$\alpha$-D-erythro-pentofuranosyl Chloride .................................. 159
      2.2.4. Improvements of Stereoselectivity for Glycosylation
         2.2.4.1. Synthesis of 6-(2-Alkylimidazol-1-yl)purines .............................................. 161
         2.2.4.2. Coupling of 6-(2-Alkylimidazol-1-yl)purines with the Chlorosugar ............... 162
         2.2.4.3. Effects of Solvent Mixtures: Polarity and Solubility .................................... 165
         2.2.4.4. Experimental Evidence for Preferential Solvation of a Purine Sodium Salt in a Binary Solvent Mixture (CH$_3$CN/CH$_2$Cl$_2$) ........................................ 171
   2.3. Attempted Glycosylation of Glucopyranose Derivatives via the Sodium Salts of 6-
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-CdA</td>
<td>2-Chloro-2’-deoxyadenosine</td>
</tr>
<tr>
<td>2’-dAdo</td>
<td>2’-Deoxyadenosine</td>
</tr>
<tr>
<td>5’NT</td>
<td>5’-Nucleotidase</td>
</tr>
<tr>
<td>ADA</td>
<td>Adenosine deaminase</td>
</tr>
<tr>
<td>BSA</td>
<td>N,O-Bis(trimethylsilyl)acetamide</td>
</tr>
<tr>
<td>DBU</td>
<td>1,8-Diazabicyclo[5.4.0]undec-7-ene</td>
</tr>
<tr>
<td>DCE</td>
<td>1,2-Dichloroethane</td>
</tr>
<tr>
<td>DCK</td>
<td>Deoxycytidine kinase</td>
</tr>
<tr>
<td>de</td>
<td>Diastereomeric excess</td>
</tr>
<tr>
<td>dr</td>
<td>Distereomeric ratio</td>
</tr>
<tr>
<td>DFT</td>
<td>Density functional theory</td>
</tr>
<tr>
<td>dGuo</td>
<td>2’-deoxyguanosine</td>
</tr>
<tr>
<td>dGK</td>
<td>Deoxyguanosine kinase</td>
</tr>
<tr>
<td>DIPEA (DIEA)</td>
<td>Diisopropylethylamine</td>
</tr>
<tr>
<td>DMA</td>
<td>N,N-Dimethylaniline</td>
</tr>
<tr>
<td>DMAc</td>
<td>N,N-Dimethylacetamide</td>
</tr>
<tr>
<td>DMAP</td>
<td>N,N-Dimethylaminopyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-Dimethylformamide</td>
</tr>
<tr>
<td>DPC</td>
<td>N,N-Diphenylcarbamoyl</td>
</tr>
<tr>
<td>EC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>50% Effective concentration</td>
</tr>
<tr>
<td>G-C</td>
<td>Group-contribution (method)</td>
</tr>
<tr>
<td>HBA</td>
<td>Hydrogen bond acceptor</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>HBD</td>
<td>Hydrogen bond donor</td>
</tr>
<tr>
<td>HMDS</td>
<td>Hexamethyldisilazane</td>
</tr>
<tr>
<td>HRMS</td>
<td>High resolution mass spectra</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>50% Inhibitory concentration</td>
</tr>
<tr>
<td>LC</td>
<td>Local composition</td>
</tr>
<tr>
<td>LRMS</td>
<td>Low resolution mass spectrum</td>
</tr>
<tr>
<td>MCC</td>
<td>Minimum cytotoxic concentration</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimum inhibitory concentration</td>
</tr>
<tr>
<td>MS</td>
<td>Molecular sieve</td>
</tr>
<tr>
<td>NMP</td>
<td>N-Methylpyrrolidinone</td>
</tr>
<tr>
<td>NOE</td>
<td>Nuclear Overhauser effect</td>
</tr>
<tr>
<td>P53</td>
<td>Tumor suppressor protein</td>
</tr>
<tr>
<td>PARB</td>
<td>Poly(ADP-ribose) synthase</td>
</tr>
<tr>
<td>PNPase</td>
<td>Purine nucleoside phosphorylase</td>
</tr>
<tr>
<td>PTC</td>
<td>Phase transfer catalyst</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>TMSOTf</td>
<td>Trimethylsilyl trifluoromethanesulfonate</td>
</tr>
<tr>
<td>TPase</td>
<td>Thymidine phosphorylase</td>
</tr>
<tr>
<td>Tol</td>
<td>p-Toluoyl (p-Methylbenzoyl)</td>
</tr>
<tr>
<td>UPase</td>
<td>Uridine phosphorylase</td>
</tr>
<tr>
<td>S.M.</td>
<td>Starting material</td>
</tr>
</tbody>
</table>
General Experimental Procedures

Uncorrected melting points were determined with a capillary tube apparatus. UV spectra were determined with solutions in MeOH unless otherwise noted. $^1$H NMR spectra were obtained with Varian 300 or 500 MHz spectrometers in CDCl$_3$ or DMSO-d$_6$ (Me$_4$Si internal). Signals are reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), quint (quintet), sext (sextet), sept (septet), br s (broad singlet), m (multiplet). Coupling constants are reported in hertz (Hz). Observed multiplicities are noted within quotation marks for $^1$H NMR peaks that should exhibit more complex splitting. $^{13}$C NMR spectra were obtained on Varian spectrometers operating at 75 MHz or 125 MHz in CDCl$_3$ or DMSO-d$_6$ with chloroform (77.4 ppm) or DMSO (40.5 ppm) as internal reference. Mass spectral data (CI, EI, FAB) were obtained from the Brigham Young University mass spectrometry facility. High-resolution mass spectra (HRMS) were determined under FAB conditions (glycerol or thioglycerol matrix) unless otherwise noted (CH$_4$ was used for CI). Peaks for $^{35}$Cl and $^{79}$Br only are given for chlorine- and bromine-containing compounds.

Reagent grade chemicals were used, and solvents (CH$_2$Cl$_2$, CH$_3$CN, DMA, DMF, DCE, pyridine and toluene) were dried over and distilled from CaH$_2$, or dried by passage through a Glass Contour solvent drying system containing cylinders of activated alumina. THF was dried by reflux over Na/benzophenone, and distilled.

Volatile materials were flash evaporated at <35 °C under house vacuum or with a mechanical oil pump vacuum in vacuo. TLC was performed with Whatman Al Sil G sheets with visualization under 254 nm light. TLCs were developed with the following solvent systems: MeOH/CH$_2$Cl$_2$ (1-33%) or SSE [EtOAc/i-PrOH/H$_2$O (4:1:2), upper
layer], or EtOAc/hexanes (30-85%). Sorbent technologies silica gel 62 (60-200 mesh) or Dowex 1 x 2 \([\text{OH}^-]\) was used for column chromatography. Solid products were dried in vacuo over \(\text{P}_4\text{O}_5\) for \(\geq 1\) day. Stable compounds were dried at elevated temperatures. 2-Deoxy-3,5-di-\(\text{O}(p\text{-toluoyl})\)-\(\alpha\)-\(\text{D-erythro}\)-pentofuranosyl chloride was dried in vacuo over solid NaOH, and stored in a refrigerator over solid NaOH/CaCl\(_2\).
List of Tables

CHAPTER 3

Table 1: Solvent effects on the S_NAr displacement of chloride from 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-2,6-dichloropurine.................................77

Table 2: Tritylation of persilylated guanine.............................................................82

Table 3: Effects of 6-halogen atom sizes on regioselectivity........................................93

Table 4: Average close contact distances <r> based on theoretical calculations........95

CHAPTER 4

Table 1: Solvatochromic parameters of some related solvents...............................145

Table 2: Dielectric constants of some solvents.........................................................146

Table 3: Stereoselectivity for glycosylation of the sodium salt of 6-(1,2,4-triazol-4-yl)purine.................................................................182

Table 4: Stereoselectivity for glycosylation of the sodium salt of 6-(imidazol-1-yl)purine.................................................................183

Table 5: Stereoselectivity for glycosylation of the sodium salt of 6-(2-propylimidazol-1-yl)purine.................................................................189

Table 6: Stereoselectivity for glycosylation of the sodium salt of 6-(2-hexylimidazol-1-yl)purine.................................................................190

Table 7: Stereoselectivity for glycosylation of the sodium salt of 6-(2-dodecylimidazol-1-yl)purine.................................................................192
CHAPTER 5

Table 1: Stereoselectivity for glycosylation of the sodium salt of 2-chloro-6-(2-propylimidazol-1-yl)purine……………………………………………………………………..236

Table 2: Stereoselectivity for glycosylation of the sodium salt of 2-chloro-6-(imidazol-1-yl)purine……………………………………………………………………..243
List of Figures

CHAPTER 1

Figure 1: Lewis acid-dependent stereoselectivity of glycosylation............................11

CHAPTER 2

Figure 1: Determination of N7 and N9 isomers by $^{13}$C NMR.................................37
Figure 2: Acetyl rearrangement in 1,3,4,6-tetra-$O$-acetylglucosamine.......................38
Figure 3: Acid-catalyzed removal of 6-$O$-DPC.........................................................42
Figure 4: Stabilization of an oxocarbenium ion by the anchimeric effect....................45
Figure 5: Mechanism of glycosylation under Lewis acid conditions..........................46
Figure 6: Approximate relative activation energies for the formation of oxocarbenium cations..............................................................................................................46
Figure 7: $^1$H NMR spectrum of compound 24.........................................................49
Figure 8: Potential energy diagram for Lewis acid-catalyzed glycosylation.................50

CHAPTER 3

Figure 1: 6-(Pyrrol-1-yl)purine acyclic nucleosides.................................................68
Figure 2: Crystal structure of 9-methyl-6-(pyrrol-1-yl)purine....................................68
Figure 3: Intermolecular hydrogen bonding and its effects on the basicity of imidazole.77
Figure 4: Possible mechanism for displacement of imidazole....................................80
Figure 5: ORTEP diagram of 9-ethyl-6-(1,2,4-triazol-4-yl)purine............................86
Figure 6: Crystal structure of 9-benzyl-6-(1,2,4-triazol-4-yl)purine............................86
Figure 7: NOESY of compound 39e............................................................................90
Figure 8: ORTEP diagram of 2-amino-9-benzyl-6-(imidazol-4-yl)purine…………………92
Figure 9: Correlation between N7/N9 alkylation regioselectivity
and 6-halogen atom sizes................................................................................................................93
Figure 10: Minimized conformational energies based on MM2 calculations.................95
Figure 11: Examples of calculated minimum conformational energies
and close contact distances.................................................................................................................96
Figure 12: Correlation between the calculated average close contact distances and
regioselectivity for N7/N9 alkylation...............................................................................................97
Figure 13: The most stable conformations of 6-heteroarylpurines.................................98
Figure 14: Steric energy barriers of 6-heteroarylpurines calculated
at the RHF/6-31 G* level..................................................................................................................99
Figure 15: Dependence of the bond length (C6–N1′) on conformations of 6-heteroarylpurines.................................................................100
Figure 16: Twisted conformation of 6-(4,5-diphenylimidazol-1-yl)purine (MM2)……102
Figure 17: NOE for 2-chloro-7/9-ethyl-6-(4,5-diphenylimidazol-1-yl)purine…………103
Figure 18: NOE difference spectra for 2-chloro-7-ethyl-6-(4,5-diphenylimidazol-1-yl)purine..............................103
Figure 19: ORTEP diagram of 6-(2-hexylimidazol-1-yl)purine..............................................104

CHAPTER 4

Figure 1: Potential energy diagrams for glycosylation.................................................148
Figure 2: N3 glycosylation of the imidazole ring.................................................................150
Figure 3: Calibration of the Varian 500 MHz instrument (EtOAc/toluene)...............154
Figure 4: Calibration of the VARIAN 500 MHz instrument with 9-[2-deoxy-3,5-di-O-(p-toluoyl)-α/β-D-erythro-pentofuranosyl]-6-(2-propylimidazol-1-yl)purines..................155

Figure 5: Effects of relaxation delays on the accuracy of measured dr values of glycosylation products of 2-chloro-6-(2-isopropylimidazol-1-yl)purine.........................156

Figure 6: Anomerization of the α chlorosugar in CDCl₃ at ambient temperature........158

Figure 7: Solvent effects (a) and effects of alkyl chains (b) on the stereoselectivity of glycosylation via the sodium salt method.........................166

Figure 8: Preferential solvation of a purine sodium salt in CH₃CN/CH₂Cl₂.............172

Figure 9: Solvatochromism of the sodium salt of 6-(2-butylimidazol-1-yl)-2-chloropurine.................................................................174

CHAPTER 5

Figure 1: Determination of the dr for glycosylation of 2-chloro-6-(2-propylimidazol-1-yl)purine (3a) in CH₃CN/CH₂Cl₂ in comparison with that of 2-chloro-6-(2-isopropylimidazol-1-yl)purine (3d) in DMF..................210

Figure 2: Effects of 6-(2-alkyl/4,5-diphenylimidazol-1-yl) substituents on the stereoselectivity for glycosylation of 2-chloropurines.................................211

Figure 3: NOE effects for 6-amino-2-chloro-9-[2-deoxy-3,5-di-O-(p-toluoyl)-D-erythro-β-D-pentofuranosyl]purine (20)..............216

Figure 4: NOE effects for compound 21.................................................217

Figure 5: NOE effects for 2-chloro-9-[2-deoxy-3,5-di-O-(p-toluoyl)-β-D-erythro-pentofuranosyl]-6-(2-propylimidazol-1-yl)purine (12a).......................218

Figure 6: ORTEP diagram of cladribine.................................................218
List of Schemes

CHAPTER 1

Scheme 1: The first synthesis of cytidine by the Hilbert-Johnson Method......................2
Scheme 2: The first synthesis of adenosine by the Fischer-Helferich method..................2
Scheme 3: The first synthesis of guanosine .................................................................3
Scheme 4: Stereoselective Vorbrüggen glycosylation......................................................7
Scheme 5: Synthesis of 2’-deoxynucleosides by the sodium salt method.........................8
Scheme 6: Anchimeric assistance of the 2’-α-benzylthio group
in acid catalyzed glycosylation..................................................................................10
Scheme 7: Anchimeric assistance of the 2’-α-phenylthio group
in glycosylation using SnCl₄.......................................................................................10
Scheme 8: Anchimeric assistance of the 2’-α-arylselenyl group.....................................11
Scheme 9: Stereoselective synthesis of pyrimidine nucleosides from a glycal...............12
Scheme 10: Stereoselective synthesis of purine nucleosides from a glycal......................12
Scheme 11: Synthesis of 2’-deoxynucleosides by photocleavage..................................13
Scheme 12: Anchimeric assistance of the 3-α-O-(2-methylsulfinyl)ethyl group............14
Scheme 13: Anchimeric assistance of the 3-α-C-methoxythiocarbonylmethyl
and 3-α-C-methoxycarbonylmethyl groups................................................................14
Scheme 14: Anchimeric assistance of the 3-O-α-(N-benzoyl)carbamoyl group............15
Scheme 15: Anchimeric assistance of the 3-O-α-thiocarbamyl group............................16
Scheme 16: Anchimeric assistance of the 3-O-(3,4,5-trimethoxybenzoyl) group............16
Scheme 17: Intramolecular glycosylation of 2’-deoxysugars
with a 5’-tethered pyrimidine....................................................................................18
Scheme 18: Intramolecular Vobrürger glycosylation of 2’-deoxysugars with a 5’-tethered pyrimidine........................................18

Scheme 19: Synthesis of a purine 2’-deoxynucleoside by intramolecular glycosylation.....................................................19

Scheme 20: 1,5-Cyclophosphate for stereoselective synthesis of α-nucleosides..........19

Scheme 21: Regioselective glycosylation with the Robins reagent.........................22

CHAPTER 2

Scheme 1: Synthesis of guanosine.........................................................36

Scheme 2: Synthesis of 9-(β-D-glucopyranosyl)guanine........................................37

Scheme 3: Preparation of 1,3,4,6-tetra-O-acetyl-2-deoxy-
2-trifluoroacetamido-α-D-glucopyranose.................................................39

Scheme 4: Coupling of the Robins reagent with 2-acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-
α-D-glucopyranose.................................................................40

Scheme 5: Preparation of 1,3,4,6-tetra-O-acetyl-2-deoxy-2-phthalimido-D-glucose and its activation.................................................................44

Scheme 6: Coupling of the Robins reagent with 3,4,6-tri-O-acetyl-1-O-
trichloroacetamidoyl-2-deoxy-2-phthalimido-β-D-glucopyranose.......................48

CHAPTER 3

Scheme 1: Synthesis of 6-(1,2,4-triazol-4-yl)purines..................................72

Scheme 2: Synthesis of 6-(imidazol-1-yl)purine (Method 1)..........................73

Scheme 3: Synthesis of 6-(imidazol-1-yl)purine (Method 2)..........................74
Scheme 4: Synthesis of 2-chloro-6-(imidazol-1-yl)purine (Method 1)………………….75
Scheme 5: Synthesis of 2-chloro-6-(imidazol-1-yl)purine (Method 2)………………….76
Scheme 6: Replacement of the 2-Cl group by imidazole in DMF……………………….77
Scheme 7: Synthesis of 2-chloro-6-(imidazol-1-yl)purine (Method 3)………………….78
Scheme 8: Attempted synthesis of 2-amino-6-(imidazol-1-yl)purine……………………79
Scheme 9: Attempted synthesis of 2-acetamido-6-(imidazol-1-yl)purine……………….80
Scheme 10: Tritylation of guanine……………………………………………………….83
Scheme 11: Synthesis of 2-amino-6-(imidazol-1-yl)purine and 2-acetamido-6-(imidazol-1-yl)purine………………………………………………..83
Scheme 12: Tritylation of 2-N-acetylguanine…………………………………………84
Scheme 13: Alkylation of 6-(1,2,4-triazol-4-yl)purine………………………………….85
Scheme 14: Alkylation of 6-(imidazol-1-yl)purine……………………...………………87
Scheme 15: Benzylation at the imidazole ring……………………………………….88
Scheme 16: Effects of deprotonation and protonation of 6-(imidazol-1-yl)purine on alkylation………………………………………………………89
Scheme 17: Alkylation of 2-amino-6-(imidazol-1-yl)purine……………………………91
Scheme 18: Alkylation of 2-chloro-(6-imidazol-1-yl)purine……………………………92
Scheme 19: Twisted conformation lowers the regioselectivity for N9 alkylations……...102

CHAPTER 4

Scheme 1: Glycosylation of 6-(imidazol-1-yl)purines with 1-O-acetyl-
2,3,5-tri-O-benzoyl-β-D-ribofuranose under Lewis acid conditions………………….150
Scheme 2: Glycosylation of 2-amino-6-(imidazol-1-yl)purine
with crude 2,3,5-tri-\(O\)-benzoyl-D-ribofuranosyl chloride by the sodium salt method....151

Scheme 3: Synthesis of
2-deoxy-3,5-di-\(O\)-(p-toluoyl)-\(\alpha\)-D-erythro-pentofuranosyl chloride..................158

Scheme 4: Stereoselectivity for glycosylation of the sodium salt of 11..................160

Scheme 5: Stereoselectivity for glycosylation of the sodium salt of 1a..................160

Scheme 6: Synthesis of 2-alkylimidazoles..........................................................161

Scheme 7: Synthesis of 6-(2-alkylimidazol-1-yl)purines........................................162

Scheme 8: Stereoselectivity for glycosylation of the sodium salt of 21c.................164

Scheme 9: Stereoselectivity for glycosylation of the sodium salt of 21a...............164

Scheme 10: Stereoselectivity for glycosylation of the sodium salt of 21b.............165

Scheme 11: Glycosylation of a glucopyranosyl bromide via the sodium salts of 6-(imidazol-1-yl)purines.................................................................175

CHAPTER 5

Scheme 1: Synthesis of 6-(2-alkylimidazol-1-yl)-2-chloropurines (Method 1)........207

Scheme 2: Synthesis of 2-chloro-6-(2-propylimidazol-1-yl)purine (Method 2).......207

Scheme 3: Synthesis of 2-alkylimidazoles by cyclization....................................208

Scheme 4: Competitive pathways for cyclizations of PhCH\(_2\)CHO.....................209

Scheme 5: Synthesis of 2-chloro-6-(imidazol-1-yl)purines (Method 3)..............209

Scheme 6: Stereoselectivity for glycosylation of the sodium salts of 2-chloro-6-(imidazol-1-yl)purines.................................................................212

Scheme 7: Activation of imidazole ring as a better leaving group by benzylation at N3.................................................................214
Scheme 8: Synthesis of 2-chloro-2’-deoxyadenosine…………………………………..214

Scheme 9: Stereoselectivity for glycosylation of the sodium salt of 6-amino-2-chloropurine…………………………………………………………………………………………………..215
Chapter 1

Introduction: Methods of Nucleoside Synthesis

There are two major approaches for nucleoside synthesis. One is direct glycosylation of natural and/or modified purine and pyrimidine bases and their derivatives; the other is construction of purine or pyrimidine rings from simple N-glycosylated precursors. Modification of the base, or sugar, or both moieties, has applications in synthesis of specific nucleosides.

In synthesis of nucleosides by the direct glycosylation, both regioisomers and stereoisomers can be formed. The most challenging obstacles involve simultaneously achieving regio- and stereocontrol of glycosylation. The history and recent improvements will be reviewed.

1. General Methods of Nucleoside Synthesis by Direct Glycosylation

1.1. Hilbert-Johnson Method

This method was used for the first synthesis of uridine and cytidine.\(^1\) 2,4-Dichloropyrimidine was treated with sodium ethoxide to give 2,4-diethoxypyrimidine, which was coupled with 2,3,5-tri-\(O\)-acetyl-D-ribofuranosyl bromide with elimination of EtBr. Ammonolysis in methanolic ammonia removed the protecting acetyl groups, and displaced the 4-ethoxy group with \(\text{NH}_2\) to give cytidine (Scheme 1). Uridine was obtained by treatment of cytidine with cytidine deaminase. The 2,4-diethoxypyrimidine structure excluded tautomeric isomerization and provided exclusive N1 glycosylation. The reaction duration was found to depend on the reactivity of the halosugar. It required 1-2 h for 2-deoxy-3,5-di-\(O\)-(p-toluoyl)-\(\alpha\)-D-erythro-pentofuranosyl chloride, several
days for 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride, and was even more protracted for peracyl-D-glucopyranosyl halides.

Scheme 1. The first synthesis of cytidine by the Hilbert-Johnson method

1.2. Fischer-Helferich Silver Salt Method and Mercury Salt Method

The first synthesis of adenosine and guanosine was accomplished by the Fischer-Helferich method. Condensation of 2,8-dichloroadenine silver salt and an acylated ribofuranosyl chloride, followed by ammonolysis and reductive dehalogenation gave adenosine (Scheme 2).

Scheme 2. The first synthesis of adenosine by the Fischer-Helferich method
To prepare guanosine, the coupling product was selectively dehalogenated at C8. Diazotization and dediazoniation/hydroxylation at C-6 followed by ammonolysis of the 2-Cl gave guanosine (Scheme 3).

Scheme 3. The first synthesis of guanosine

The applicability of that method for nucleoside synthesis was limited because of its low yield, which was significantly improved by using a chloromercuropyridine salt of a base (30-40%). The use of mercury salts of purine derivatives gave the nucleosides in relatively good yields. However, the mercury method is not used for the preparation of clinical reagents due to the difficulty in removing the toxic mercury salts.

1.3. Fusion Method

This method was introduced by Sato et al. in 1960. Heating an acylated sugar and a purine (pyrimidine) base in the presence of an acid catalyst at reduced pressure gave condensation products (after melting). The reaction usually gave anomeric mixtures. The first synthesis of 2-chloro-2′-deoxyadenosine (cladribine, 2-CdA) as a target compound was accomplished using a fusion method. Coupling of 2,6-dichloropurine with 1,3,5-tri-
$O$-acetyl-$2$-deoxy-$\alpha$-$D$-erythro-pentofuranose gave an anomeric mixture. Fusion of $2,6$-dichloropurine with methyl $2$-deoxy-$3,5$-di-$O$-($p$-toluoyl)-$D$-erythro-pentofuranoside also gave a mixture of both anomers, which was separated by silica gel chromatography.

Ammonolysis of the $6$-$Cl$ group and simultaneous deprotection of the toluoyl groups gave $2$-$CdA$ with a total yield of $8\%$. It was shown that coupling of $1,3,5$-tri-$O$-acetyl-$2$-deoxy-$\alpha$-$D$-erythro-pentofuranose with purines gave a mixture of $\alpha/\beta$-anomers in moderate yields. Coupling of $2,6$-dichloro-$1$-deaza-$9$-$H$-purine with a $3$-deoxyribose derivative by the acid-catalyzed fusion method gave an anomeric mixture of the $N9$-$\alpha/\beta$-$3'$-deoxynucleosides ($29\%$ and $52\%$, respectively). Pedersen et al. reported their synthesis of pyrimidine $1'$-aza-$C$-nucleosides by fusion of $5$-bromouracil, $5$-bromocytosine and $5$-bromoisocytosine with ($3R,4R$)-$4$-(hydroxymethyl)pyrrolidin-$3$-ol in $40$-$41\%$ yield.

1.4. Transglycosylation Method

Transglycosylation can occur both intermolecularly and intramolecularly. Intramolecular $N3 \to N9$ and $N7 \to N9$ transglycosylations were observed in nucleoside synthesis. Seela et al. reported a thermal $N9 \to N7$ isomerization of $2$-$N$-acetyl-$2'$-deoxyguanosine in a melt and in solution, and a zwitterionic $7,9$-bis($2$-deoxy-$D$-erythro-pentofuranosyl)guanine intermediate was proposed. The overall intramolecular transglycosylation was suggested to occur by an intermolecular reaction, instead of a direct migration of the sugar moiety. Intermolecular transglycosylation is a useful method for preparation of complicated nucleosides. The transglycosylation reaction proceeds with high stereoselectivity for the $\beta$-anomer if the glycosyl donor contains a $2$-$\alpha$-acyloxy group. Otherwise, the reaction usually gives anomeric mixtures as in direct
glycosylation. Attempts to prepare 2'-O-methyladenosine by transglycosylation of 3',5'-di-O-acetyl-2'-O-methyluridine, or 4-N-acetyl-3',5'-di-O-acetyl-2'-O-methylcytidine with persilylated 6-benzoyladenine were not very successful, and gave β-anomers in yields of 30% and 50% respectively, with significant amounts of α-anomers.¹⁸ A method for synthesis of 2'-deoxynucleosides via transglycosylation of 6-oxopurine ribonucleosides followed by radical reductive deoxygenation was developed.¹⁹ Transglycosylation of 2'-O-acetyl-3',5'-O-(tetraisopropyl-1,3-disiloxanediyl)inosine with 6-methylpurine in chlorobenzene with 0.1 equivalent of p-toluenesulfonic acid was reported to proceed quantitatively.¹⁹

1.5. Enzymatic Synthesis

Two types of enzymes have been used, i.e., trans-N-deoxyribosylases and nucleoside phosphorylases. Trans-N-deoxyribosylases were used to prepare biologically active nucleosides such as cladribine²⁰ in spite of their limited sources and applications. The application of phosphorylases was described earlier. Krenitsky ²¹ had noted the enzymatic equilibrium-transfer reaction of the sugar moiety of one nucleoside to a different heterocyclic base. Purine nucleoside phosphorylases (PNPase) catalyze the equilibrium-transfer reaction between purine nucleosides and purines, and uridine or thymidine phosphorylases (UPase or TPase) in the case of pyrimidine nucleosides and pyrimidines. Transglycosylations using these enzymes as catalysts have recently been applied to the synthesis of biologically active purine nucleosides. Originally, the method required two enzymes, i.e., a UPase or TPase which provides the glycosyl donor as a furanosyl-1-phosphate, and a PNPase which catalyzes the formation of the glycosyl bond between a purine and furanosyl-1-phosphate.²² Wong et al. developed an enzymatic
procedure employing only PNPase with either $N_7$-methylinosine or $N_7$-methylguanosine as the ribosyl donor. $N_7$-methylation gave rise to an essentially irreversible transglycosylation.\textsuperscript{23} Recently, whole bacteria cells containing UPase and/or TPase and PNPase were used as biocatalysts for transglycosylation to purines.\textsuperscript{24-29} 2,6-Diamino-9-(3-deoxy-$\beta$-D-erythro-pentofuranosyl)purine was prepared from 3’-deoxycytidine by an enzymatic reaction cascade with whole bacterial cells $(E. \text{Coli} \text{ BMT-4D/1A})$.\textsuperscript{24,25} 3’-Deoxycytidine was deaminated to 3’-deoxyuridine by whole cell $(E. \text{Coli} \text{ BM-11})$ cytidine deaminase. Phosphorolytic cleavage of 3’-deoxyuridine by UPase with inorganic phosphate gave 3-deoxy-$\alpha$-D-erythro-pentofuranosyl-1-phosphate. Coupling the glycosyl phosphate with 2,6-diaminopurine by PNPase gave the nucleoside in a yield of 72%. The whole bacterial cell $(E. \text{Coli} \text{ BMT-4D/1A})$ PNPase was also used to prepare cladribine by glycosyl transfer from excess 2’-deoxyguanosine (dGuo) to 2-chloroadenine.\textsuperscript{26} The transfer reaction was driven to completion by removal of 2-CdA.

1.6. Vorbrüggen Glycosylation (Silyl-Hilbert-Johnson Method)

The Vorbrüggen glycosylation\textsuperscript{14,30,31} and its variations involving the reaction of persilylated bases with glycosyl donors under Lewis acid conditions have been used to prepare many modified nucleosides. This method is particularly useful when there is an $\alpha$-acyloxy group at C-2 of the glycosyl donor, producing $\beta$-nucleosides in a stereocontrolled manner because of the neighboring group participation (Scheme 4). In the case of 2’-deoxynucleosides, however, no such participation is present; thus, anomeric mixtures of varying ratios result which are often difficult to separate. For purines, there is also a problem of regioselectivity as in the transglycosylation methods.
Scheme 4. Stereoselective Vorbrüggen glycosylation

1.7. Sodium Salt Method

The sodium salt method has been utilized for the synthesis of acyclic, furanosyl and pyranosyl nucleosides.\(^1\) A sodium salt of a purine was formed in situ, and treated with a halosugar as the glycosyl donor. For the synthesis of 2'-deoxynucleosides, stereospecific formation of the β-isomer requires the S\(_{N2}\) pathway completely with exclusion of anomerization of the α-chlorosugar.

Due to the availability of crystalline 2-deoxy-3,5-di-\(O\)-(p-toluoyl)-\(α\)-D-\(erythro\)-pentofuranosyl chloride, many groups have effected coupling of this sugar derivative with different bases via S\(_{N2}\) displacement of chloride. Earlier studies claimed that glycosylation of the sodium salt of acidic heterocycles with this sugar chloride in CH\(_3\)CN at ambient temperature gave exclusively β-anomer, or β-anomers of regioisomers (N9/N7 for a purine).\(^{32-37}\) Coupling of this protected 2-deoxy chlorosugar with the sodium salt of 6-chloropurine and 2,6-dichloropurine was reported to give a mixture of two β-isomers, the N7 and N9 nucleosides (Scheme 5).\(^{32}\)
Scheme 5. Synthesis of 2'-deoxynucleosides by the Sodium Salt Method

The reaction was also tried with 2,6-dibromopurine, 2,6-bis(methylthio)purine, 6-chloro-, 6-bromo- and 2-bromo-6-methylthiopurine. As to the latter four purine bases, dominant N9-β-glycosylation products (50-60%) were obtained with minor N7-β-products (10-15%) and traces of N7 and N9-α-products. However, glycosylation of 2,6-dibromopurine gave the N9-β-nucleoside (48%) as the major product with minor N9-α- (12%), N7-β- (6.6%) and N7-α-products (3.8%). The differences were presumed to be caused by substituent-induced differences in reaction rates, i.e., a slower rate in N9-glycosylation of 2,6-dibromopurine, steric effects of the 6-bromo group and anomeration of the α-chlorosugar.

This protected α-chloro-2-deoxysugar was also coupled with pyrimidine bases. Both anomers were usually produced, and the ratio depended on solvent, catalyst, and base structures. The best results were obtained with CHCl₃ as the reaction medium. The coupling reaction of 2,4-bis(trimethylsilyloxy)-(E)-5-(2-bromovinyl)uracil gave only the β-anomer with a yield of 72%. For both thymine(TMS)₂ and uracil(TMS)₂, only β-isomers were formed in CHCl₃ in high yield (83%, 92%), but the stereoselectivity was poor for glycosylation of cytosine(TMS)₂. When the chlorosugar was allowed to
anomerize in CH$_3$CN before addition of the base, the $\alpha$-nucleoside was produced exclusively.

Attempted couplings with guanine bases were ineffective.$^{37,41}$ 2-Amino-6-chloropurine gave a mixture of $\beta$-isomers ($9\beta/7\beta \sim 3:1$). Protection of the NH$_2$ group with an N,N-dibutylformamido group gave an even poorer result ($9\beta/7\beta/3\beta \sim 1.0:0.9:0.5$) with the N9-$\beta$-nucleoside as the major product (29%). The formation of $\alpha$-anomers was not mentioned. Coupling of 2-amino-6-methoxypurine with the $\alpha$-chlorosugar gave a regioisomeric mixture of $\beta$-anomers (N9/N7, 2:1) in a yield of 72%.

The method was also adapted to glycosylation of heterocyclic systems with 2,3,5-tri-$O$-benzyl-$\alpha$-D-arabinofuranosyl chloride$^{34-36}$ and 2,3-$O$-isopropylidene-$5-O$-($t$-butyldimethylsilyl)-$\alpha$-D-ribofuranosyl chloride.$^{36}$

2. Strategies for Stereoselective Glycosylation of 2-Deoxy-D-erythro-pentofuranose Derivatives

As shown above, glycosylation of the sodium salts of heterocyclic bases with crystalline 2-deoxy-3,5-di-$O$-($p$-toluoyl)-$\alpha$-D-erythro-pentofuranosyl chloride via the SN2 pathway is a convenient and efficient method for synthesis of 2’-deoxynucleosides, although the stereoselectivity varies with the structure of the bases and reaction conditions. In recent attempts for Lewis acid catalyzed glycosylation, the most dominant concept hinges on 1,2-trans glycosylation with temporarily installed 2-$\alpha$ substituents such as benzylthio,$^{42}$ phenylthio,$^{43}$ phenylselenenyl,$^{44}$ or iodo$^{45,46}$ functioning as neighboring groups, followed by removal of such groups via reduction or elimination. An alternative with 3-$\alpha$ substituents is generally less successful.$^{47-51}$
2.1. Anchimeric Assistance of 2-α-Substituents

An earlier application of this strategy was the synthesis of purine 2’-α-thio-2’-deoxynucleosides (Scheme 6). Both the yields and selectivity were poor.

![Scheme 6. Anchimeric assistance of the 2'-α-benzylthio group in acid catalyzed glycosylation](image)

Scheme 6. Anchimeric assistance of the 2'-α-benzylthio group in acid catalyzed glycosylation

However, when the Lewis acid was switched to SnCl₄, the coupling reaction with silylated thymine, uracil and 4-N-acetylcytosine gave very good selectivity for the β-isomer (Scheme 7). Since the phenylthio derivatives worked by a Lewis acid-dependent
mechanism, it was proposed that both episulfonium ions and thiophenyl-SnCl$_4$ complexes were involved (Figure 1).

![Figure 1. Lewis acid-dependent stereoselectivity of glycosylation](image)

1-$O$-Acetyl-2,3-dideoxy-2-$\alpha$-phenyselenyl-D-erythro-pentofuranose was coupled with silylated thymine to give high stereoselectivity (99:1) favoring the $\beta$-isomer (Scheme 8).$^{44}$ An episelenium ion, which provides the $\beta$ selectivity, was thought to be formed.

![Scheme 8. Anchimeric assistance of the 2'-$\alpha$-aryseselenyl group](image)

Variations of this methodology have involved in situ sulfenylation, selenenylation, or iodination/glycosylation of furan glycals.$^{45,46,52-55}$ A 1-chloro-2-phenylthiosugar, prepared in situ from the glycal and PhSCl, was coupled with silylated uracil to give the 1,2-\textit{trans} $\beta$-isomer of the nucleoside as the major product (Scheme 9).$^{52}$

11
Scheme 9. Stereoselective synthesis of pyrimidine nucleosides from a glycal

The ratio of products was not dependent on temperature. The reaction was studied more systematically by Liotta and coworkers. It was observed that SnCl$_4$ was a better Lewis acid than TMSOTf, as reported before. In comparison, no significant difference between phenylsulfenyl chloride and 2,4,6-triisopropylphenylsulfenyl chloride was observed. The coupling reaction with 6-chloropurine suffered from N9 versus N7 and α- versus β-glycosylation. Using TMSOTf as Lewis acid, poor stereoselectivity ($\beta/\alpha \sim 5:1$) was obtained (Scheme 10), and on the other hand, poor regioselectivity resulted using SnCl$_4$. In spite of the possible isomerization equilibrium to improve the N9 isomer, the reaction was poor.

Scheme 10. Stereoselective synthesis of purine nucleosides from a glycal
Another variation of 2-α-neighboring group participation was the use of a ribose derivative. The 2-hydroxyl group was first transformed into an acyloxy group capable of both directing glycosylation and easy photocleavage or removal by radical reduction (Scheme 11).

![Scheme 11. Synthesis of 2'-deoxynucleosides by photocleavage](image)

2.2. Anchimeric Assistance of 3-α-Substituents

The 3-α-hydroxyl group in a 2-deoxysugar can be used to tether a suitable function, which can participate in glycosylation reactions. This strategy assumes that a 3-O-directing group can block the α-face of the sugar, and hence β-nucleosides might form stereoselectively.

A 3-O-(2-methylsulfinyl)ethyl group was first used as a guiding group. It provided good stereoselectivity for β-isomer formation when a 1-O-acetyl-2-deoxy-D-erythro-pentofuranose was coupled with silylated thymine and uracil (Scheme 12). However, it was not as successful when coupled with silylated purine and cytosine (α/β~1:3) bases, or with a zinc salt of 6-piperidinopurine [α/β~23:77, 78%].
Scheme 12. Anchimeric assistance of the 3-α-O-(2-methylsulfinyl)ethyl group

Higher stereoselectivity was observed when a 3-C-methoxythiocarbonylmethyl sugar was coupled with persilylated cytosine pre-complexed with SnCl₄ (α/β, 4:96). On the other hand, when a 3-C-methoxycarbonylmethyl sugar was used, medium selectivity was obtained (α/β, 1:3) (Scheme 13). The stereoselectivity was explained by an equilibrium between oxocarbenium and bicyclic ions. The method is not general with respect to a natural sugar moiety.

Scheme 13. Anchimeric assistance of the 3-α-C-methoxythiocarbonylmethyl and 3-α-C-methoxycarbonylmethyl groups
A third approach involved a 3-O-(N-benzoyl)carbamoyl group. Phenyl 3-O-(N-benzoyl)carbamoyl-2-deoxy-1-thio-D-erythro-pentofuranoside was coupled with persilylated pyrimidines to give poor to good stereoselectivity (uracil, $\beta/\alpha \sim 6.5:1$; 4-N-acetylcytosine, 2.4:1; thymine, 14:1) (Scheme 14). An ion pair at the $\alpha$-face of the oxocarbenium ion was proposed for the improved stereoselectivity.

Scheme 14. Anchimeric assistance of the 3-O-$\alpha$-(N-benzoyl)carbamoyl group

The stereoselectivity was further improved using a thiocarbamate derivative as a glycosyl donor. 1-O-Acetyl-5-O-benzoyl-2-deoxy-3-diethylthiocarbamoyl-D-erythro-pentofuranose was coupled with silylated thymine ($\alpha/\beta \sim 4:96$, 90%) and uracil ($\alpha/\beta \sim 6:94-5:95$, 94-96%) with very high stereoselectivity (Scheme 15). Good selectivity was also achieved with 4-N-benzoylcytosine ($\beta/\alpha$, 89:11, 71%). An NMR study supported the formation of an iminium ion (57).
Scheme 15. Anchimeric assistance of the 3-O-α-thiocarbamyl group

A recent report, using 3-O-(3,4,5-trimethoxybenzoyl)-2-deoxy-D-erythro-pentofuranosyl diethyl phosphite, gave good selectivity (Scheme 16).\textsuperscript{51} Coupling reactions with 5-substituted-uracils (I, F, CF\textsubscript{3}) gave good yields and selectivity.

Scheme 16. Anchimeric assistance of the 3-O-(3,4,5-trimethoxybenzoyl) group

2.3. 5’-Tethered Bases for Intramolecular Glycosylation

Intramolecular glycosylation of 2’-deoxysugars with 5’-tethered bases is another interesting strategy for stereoselective synthesis of nucleosides. However, such base-sugar hybrids usually are not easy to prepare. Two successful examples involved 2,5’-anhydropyrimidine nucleosides.\textsuperscript{57,58}
In one of these examples, the hybrid was prepared by SNAr substitution of the 2-Cl group of 2-chloro-4-methoxypyrimidine with the 5-hydroxyl group of the sugar moiety (Scheme 17). Intramolecular N-glycosylation, followed by hydrolysis with 1 N NaOH/H₂O gave 82% of the β-nucleoside and 4% of the undesired byproduct.

In the other example, the hybrid was prepared by SNAr substitution between 2-(methylthio)-3-(benzyloxymethyl)-4-pyrimidinone and the potassium salt of 3-O-methylribose. After silylation of the base, an intramolecular Vobrüggen reaction, followed by hydrolysis with 1 N NaOH/H₂O, gave 43% of the desired β-nucleoside (Scheme 18).

A 5’-sulfur-linked pyrimidine-sugar hybrid was used to prepare 2,2’,5’-trideoxynucleosides. However, this route cannot be used to prepare simple 2’-deoxynucleosides.

This strategy has rarely been applied to purine 2’-deoxynucleosides. A 5’,8-S-anhydropurine nucleoside was used as an intermediate to prepare 6-aminopurine nucleosides with various sugar moieties (Scheme 19). Formation of α-anomer was not mentioned.
Scheme 17. Intramolecular glycosylation of 2'-deoxysugars with a 5'-tethered pyrimidine

Scheme 18. Intramolecular Vobrüggen glycosylation of 2'-deoxysugars with a 5'-tethered pyrimidine
Scheme 19. Synthesis of a purine 2'-deoxynucleoside by intramolecular glycosylation

2.4. Miscellaneous Methods

A related stereoselective synthesis of α-nucleosides is also interesting. A 1,5-cyclophosphate blocks base attack from the β-face to give dominant formation of an α-isomer (Scheme 20).\textsuperscript{61} α-Adenosine was synthesized with high stereoselectivity (30%, NMR pure). The drawback of the method lies in O-glycosylation of pyrimidine bases due to the formation of Sn-O instead of Sn-N activated species.

Scheme 20. 1,5-Cyclophosphate for stereoselective synthesis of α-nucleosides
3. Strategies for Regioselective Alkylation and Glycosylation of Purines

Alkylation and glycosylation of purines are rarely regiospecific, and give rise to mixtures of N9 and N7 products. Alkylation of the adenine anion with alkyl mesylates in DMF at 80 °C in the presence of 18-crown-6 afforded both N9 and N7 isomers in yields of 61% and 9%, respectively. Glycosylation of persilylated 6-N-benzoyleadenine with a dioxabicyclo[3.2.1]octane at ambient temperature gave both N9 and N7 products, and it was suggested that the N7 isomer was the kinetic product. It was converted into the N9 isomer at elevated temperature in the presence of TMSOTf. Glycosylation of persilylated 2-N-isobutyrylguanine with glycosyl donors at room temperature with TMSOTf gave mixtures of N9 and N7 products (1:1), and higher reaction temperatures in the presence of TMSOTf did not convert N7 to N9 isomers. Coupling of persilylated 2-N-acetylguanine with acetylated glucosamine gave a 2:1 mixture of N9 and N7 nucleosides. Condensation of guanine or 2-N-acetylguanine with 1,3-dibenzzyloxy-2-chloromethoxypropane using various condensation catalysts [SnCl₄, Hg(CN)₂, etc.] gave both N9 and N7 products. Alkylation of 2-amino-6-chloropurine with cyclopropylmethyl mesylate or chloride in the presence of K₂CO₃ in DMF gave a mixture of N9 and N7 products (6.5:1 and 7.0:1 respectively) in a combined yield of 78%. Vorbrüggen glycosylation of 2-N-acetylguanine or 2-amino-6-chloropurine with 2-acetoxy-4-benzyoxymethyltetrahydrofuran in DMF or NMP gave both N9 and N7 isomers with a ratio that depended on reaction conditions. Debart et al. coupled silylated 2,6-dichloropurine with 1-O-acetyl-3,5-di-O-(p-toluoyl)-2-deoxy-D-erythro-pentofuranose in the presence of KI/18-crown-6 in CH₃CN/toluene to give a mixture of N7 (15%) and N9 (32%) isomers. Jähne et al. reported that condensation of persilylated 2-N-acetylguanine
with 1,3-diisopropoxy-2-methylthiomethoxypropane and 2-acetoxymethoxy-1,3-
diisopropoxypropane in CH$_3$CN at room temperature with a five-fold excess of SnCl$_4$
gave a 1:1 mixture of N7 and N9 isomers in good overall yield (80%). Persilylated 2-
acetamido-6-chloropurine was coupled with these alkylating reagents in DCE in the
presence of TMSOTf (1-1.2 eq.) to give the N7 products in isolated yields of 65%.$^{69}$

3.1. 6-Functional Groups for Regioselective N9 Alkylation and

**Glycosylation of Purines**

The Saneyoshi and Satoh method of glycosylation with SnCl$_4$ is usually used for
regioselective glycosylation of adenine.$^{70,71}$ Yields of $\geq 75\%$ are accessible. With 2-
aminopurines, the ratio of N9 to N7 alkylation was influenced by the size of the 6-
substituents on the purine ring, and larger groups at C-6 lead to increased ratios.$^{72}$ Bulky
protecting groups installed on C6 substituents were used to improve the selectivity of N9
alkylation.$^{73}$ For example, Geen et al. studied the effects of 6-substituents systematically.
Variation of 6-functional groups was tried to improve the N9/N7 regioselectivity of
glycosylation. They found the ratio of N9 to N7 alkylation ranged from 1.8:1 (6-
methoxy-2-aminopurine) to 25:1 (6-isopropyl-2-aminopurine), using K$_2$CO$_3$ as the base
in DMF.$^{72}$

Reese et al. reported a highly regioselective N9 alkylation of 2-amino-6-[(4-
chlorophenyl)sulfanyl]purine in the presence of K$_2$CO$_3$ in DMF with 4-acetoxy-3-
acetoxymethylbutyl mesylate (89:11 regioselectivity for N9 product in 80% yield).$^{73}$
Lower regioselectivity was observed for 2-amino-6-chloropurine (N9/N7, 82:18).

Robins et al.$^{74}$ and Kovacs et al.$^{75}$ had achieved highly regioselective N9
alkylations and glycosylations using the 6-0-diphenylcarbamoyl (6-O-DPC) protecting
group (Scheme 21). Using this Robins reagent, Lazrek et al. achieved a good regioselectivity for N9-alkylation with (2-acetoxyethoxy)methyl bromide using 18-crown-6 as the PTC and potassium t-butoxide in DMF at 0 °C (80%). Alkylation of 2-acetamido-6-O-(diphenylcarbamoyl)purine with dimethylitaconate by conjugate addition in DMF (K₂CO₃) was also highly regioselective; in contrast, alkylation of 6-N-benzoyladenine gave a mixture (N9/N7, 87:13).

Scheme 21. Regioselective glycosylation with the Robins reagent

However, Tsuji et al. reported a poorer regioselectivity for 2-amino-6-O-diphenylcarbamoyl than for 2-amino-6-chloropurine. Breipohl et al. reported highly regioselective N9 alkylation of 2-acetamido-6-O-(diphenylcarbamoyl)purine by methyl bromoacetate in DMF with DIEA as base (71%), but relatively poor regioselectivity was observed by later studies (86%, N9/N7, 71:15). Alkylation of 2-acetamido-6-O-(diphenylcarbamoyl)purine with 2-(t-butyldiphenylsilyloxy)methylallyloxymethyl acetate under Vorbrüggen conditions gave only the N7 isomer in a low yield (24%), and the same product was obtained at 85 °C, which did not isomerize to the N9 isomer. Trost et al. prepared carbanucleosides via Pd(0)-catalyzed allylic alkylation using 2-N-acetyl-6-O-DPCguanine. The alkylation gave N9 and N7 monoalkylated, and a byproduct containing two N9 alkylated 6-O-DPC-2-N-acetylguanine units with a high combined regioselectivity (N9/N7, 13:1), which depended on the ligands and reaction conditions.
Glycosylation under Vorbrüggen conditions with 2-acetamido-6-O-(diphenylcarbamoyl)purine and 2-N-trifluoroacetyl-1,3,4,6-tetra-O-acetyl-2-deoxy-β-glucosamine gave reversed overall regioselectivity (N9/N7, 6:14).\(^{81}\)

Benner et al.\(^{82,83}\) introduced 2-N-isobutyryl-6-O-[2-(p-nitrophenyl)ethyl]guanine for regioselective N9 alkylation and glycosylation. Coupling of persilylated 2-N-isobutyryl-6-O-[2-(p-nitrophenyl)ethyl]guanine with ribose homologues in the presence of TMSOTf (5-10 mol\%) at room temperature gave N9 glycosylated anomeric mixtures (60-70%). Alkylation of this purine derivative under Mitsunobu conditions was reported to give high N9 regioselectivity.

### 3.2. Thermodynamic Control versus Kinetic Control

Based on different thermodynamic stabilities and formation rates, regiocontrolled synthesis of N7 and N9 nucleosides have been partially realized.\(^{84}\) Under kinetic conditions (CH\(_3\)CN, SnCl\(_4\), room temperature), the coupling between 1,2,3,4,6-penta-O-acetyl-α-D-glucopyranose with 2-N-acetylguanine gave the N7-nucleoside (61%). When 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose was used under the same condition, 95:1 regioselectivity for the N7-nucleoside was achieved (78%). However, although not as selective as for N7, the N9-nucleosides can be formed regioselectively under thermodynamic conditions. When the reaction was performed with TMSOTf in refluxing DCE, N9/N7 ratios of about 8:1 for 1-O-acetyl-2,3,4,6-tetra-O-benzoyl-α-D-glucopyranose were obtained in a yield of 56%, and ~6:1 for 1-O-acetyl-2,3,5-tri-O-acetyl-β-D-ribofuranose (79%). Dudycz and Wright\(^{85}\) monitored the coupling reactions between the persilylated 6-oxopurines, i.e., 2-bromohypoxanthine, guanine and 2-N-(p-butylphenyl)guanine, with tetra-O-acetyl-β-D-ribofuranose in CH\(_3\)CN in the presence of
TMSOTf. The N7 β-isomer was formed first, and was converted to the N9 β-isomer via an N7,N9-bis(ribofuranosyl)nucleoside intermediate. Finally the N9 β-isomer was obtained as the major product.

Kumar et al.\(^6\) discovered that alkylation of 2-N,9-diacetylguanine with the diacetate of 2-oxa-1,4-butanediol at 105 °C for 80 h gave 2-N-acetyl-9-(2-acetoxyethoxymethyl)guanine in high yield (95%), and that alkylation at 10-15 °C in AcOH in the presence of TiCl\(_4\) for 5-6 h gave the N7 isomer in good yield (65%). It was suggested that N7 → N9 isomerization was almost irreversible in the absence of acid, and both N7 and N9 products were formed at the beginning. Isomerization of N7 ↔ N9 was faster under acid catalyzed conditions, and only the N7 product was formed initially.

4. Conclusions and Prospects

Regio- and stereoselective methods for glycosylation of purine derivatives with 2-deoxysugars are needed. Lewis acid-catalyzed coupling reactions (Vorbrüggen) require a pre-installed sugar participating function for good stereoselectivity; but the problem of regioselectivity remains. The sodium salt method can provide high stereoselectivity in less polar solvents, but the problem of regioselectivity persists. Low polarity solvents limit applications of this method with the more polar nucleic acid bases.

Several 6-functional groups have been introduced, with 6-O-DPC as the most effective. However, the labile 6-O-DPC group has been shown to be less effective for small electrophiles and for acidic glycosyl donors under Lewis acid conditions. Therefore, better 6-functional groups to exclude N7 alkylation and glycosylation are needed.
The purposes of this research include: (1) To extend the use of 2-acetamido-6-O-(diphenylcarbamoyl)purine to aminosugars, e.g., glucosamine. (2) To develop new 6-functional groups for regiospecific N9 alkylation of purines and apply this strategy for synthesis of new derivatives. (3) To apply new functional groups to the sodium salt method for regioselective and stereoselective glycosylation of purines with 2-deoxysugars. (4) To apply the developed sodium salt method for synthesis of cladribine, an antileukemia drug.
5. References and Notes


Chapter 2

Glycosylation of 2-N-Acetyl-6-O-(diphenylcarbamoyl)guanine with Ribofuranose and Glucopyranose Derivatives with Lewis Acid Catalysts

1. Introduction

An α 2-O-acyl or aroyl function on a sugar derivative usually ensures the stereoselective formation of β-nucleosides. However, regioselectivity of alkylation and glycosylation of guanine has been a persistent problem in nucleoside synthesis. It was generally accepted that purine bases are initially glycosylated at N3, and the resulting intermediate undergoes an irreversible N3 → N9 transglycosylation to give the thermodynamically more stable N9-regioisomer. With hypoxanthine and guanine, however, a fully reversible N7 → N9 transglycosylation occurs.

Vorbrüggen and coworkers reported the sole formation of guanosine by coupling persilylated 2-N-acetylguanine under Lewis acid conditions. However, the reaction gave some N7-glycosylation product as shown by later studies.

The 6-O-(diphenylcarbamoyl) (DPC) function was introduced as a protecting group for synthesis of oligonucleotides and mimics. Robins and coworkers utilized this large functional group to achieve regiospecificity for N9-glycosylation of persilylated 2-N-acetyl-6-O-(diphenylcarbamoyl)guanine. They reported the N9 glycosylation product was both the thermodynamic and kinetic product. The strategy has been applied to numerous guanine nucleoside and acyclic nucleoside syntheses using
different condensation methods. The stability of 2-N-acetyl-6-O-DPCguanine in basic conditions has been studied. It is very stable in dry diisopropylamine (t_{1/2}, >16 h), less stable in 0.1 N NaOH in H_{2}O/dioxane (v/v, 1:1) (t_{1/2}, 4 h) and much less stable in concentrated NH_{3}/H_{2}O/MeOH/i-PrOH/dioxane (t_{1/2}, 1.5 h).^{24} Alkylation under basic conditions gave good to excellent yields of N9 isomers.^{25a,b} Alkylation of the reagent under Mitsunobu^{26a-c} and Michael addition^{26d,e} conditions gave N9 isomers without mention of the formation of N7 isomers. Numerous condensations of 2-N-acetyl-6-O-DPCguanine using Lewis acids (TMSOTf, SnCl_{4}, etc.) have been reported to give N9 products regiospecifically. A few exceptions suggested the formation of small amounts of N7 isomers, which were not separated.^{27a-c} Condensations of persilylated 2-N-acetyl-6-O-DPCguanine with furanosyl acetates using Lewis acids (TMSOTf, SnCl_{4}, etc.) gave N9 nucleosides with good regioselectivity.^{27d-h} Condensations of persilylated 2-N-acetyl-6-O-DPCguanine with pyranosyl acetates using Lewis acids (TMSOTf, SnCl_{4}, etc.) also gave N9 nucleosides without noted formation of N7 nucleosides, in spite of the longer reaction times and variation of reaction conditions.^{27i-k} Coupling of the reagent with dioxane and isoxazolidine derivatives gave anomeric mixtures of N9 nucleosides with low to good yields, and the formation of N7 nucleosides was not mentioned.^{27l-n} The instability of the oxocarbenium ion derived from an isoxazolidine derivative requires strenuous reaction conditions.^{27n} Coupling of a novel glycosyl donor, (1R,2R,5R,6S)-2-acetoxy-6-acetoxymethyl-3-oxobicyclo[3.1.0]hexane with persilylated 2-N-acetylguanine in CH_{3}CN gave a mixture of N9/N7 isomers (1.5:1), whereas the N9 isomer was obtained exclusively with 2-N-acetyl-6-O-DPCguanine.^{27o} The bulky 6-O-DPC group was interestingly used to separate an N9 isomer from the N7 isomer, because the N7 isomer
did not react with diphenylcarbamoyl chloride.\textsuperscript{28} A peculiar “migration” of the 6-\textit{O}-DPC group to N2 was observed during the condensation of persilylated 2-N-acetyl-6-\textit{O}-DPCguanine with peracetyl-\textbeta-D-galactopyranose, but only the N9 isomer was isolated.\textsuperscript{29} Therefore, the 6-\textit{O}-DPC group is very effective in excluding N7 glycosylation.

However, when this Robins reagent was applied to the synthesis of a 2-amino-2-deoxyglucopyranosyl nucleoside, surprising results were obtained. Cheung et al.\textsuperscript{30} reported the coupling of 1,3,4,6-tetra-\textit{O}-acetyl-2-deoxy-2-trifluoroacetamido-\textbeta-D-glucopyranose and persilylated 2-N-acetyl-6-\textit{O}-(diphenylcarbamoyl)guanine with TMSOTf in toluene gave low yields of an N7, N9 isomeric mixture, and an analogous reaction in DCE gave only the N7 product. They suggested that the coupling reaction was irreversible and under kinetic control. The different reaction rates in toluene and DCE were presumed to cause the different regioselectivity for N9 glycosylation, which was in contrast to Robins’ results for coupling with pentofuranoses.

Isomerizations of N7 to N9 nucleosides under Lewis acid conditions has long been known, and transglycosylation allows mutual conversions between nucleosides.\textsuperscript{31,32} The kinetics of glycosylation might be controlled by the stability of the rate-determining reaction intermediate, the sugar oxocarbenium ion. The poor regioslelectivity for N9 glycosylation might be caused by the acidity of the glycosyl donor, which may cleave the 6-\textit{O}-DPC function. Therefore, coupling reactions between 2-N-acetyl-6-\textit{O}-(diphenylcarbamoyl)guanine and ribofuranose, glucopyranose and 2-amino-2-deoxyglucopyranose derivatives were studied.
2. Results and Discussion

2.1. Glycosylation of 2-N-Acetyl-6-O-(diphenylcarbamoyl)guanine with 1-O-Acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose

2-N-Acetyl-6-O-(diphenylcarbamoyl)guanine was prepared according to the reported procedure. Acetylation of guanine in DMAc gave 2-N,9-diacetylguanine (87%). Further acylation with Ph₂NCOCl in pyridine gave 2-N,9-diacetyl-6-O-(diphenylcarbamoyl)guanine, which gave 2-N-acetyl-6-O-(diphenylcarbamoyl)guanine after selective removal of the 9-acetyl group in boiling EtOH/H₂O (81%).

This reagent was persilylated (BSA, DCE, 80 °C), and coupled with 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose in toluene with TMSOTf to give protected 2-N-acetyl-9-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-6-O-(diphenylcarbamoyl)guanine (96%) as the only regiosiomer. The reaction was complete in 1 h. More extended reaction times caused partial loss of the 6-O-diphenylcarbamoyl group (presumably hydrolysis by adventitious H₂O) as shown by the appearance of a lower spot on TLC. The coupling product was deprotected in NH₃/H₂O/MeOH at 60 °C to give guanosine (67%) (Scheme 1).
Scheme 1. Synthesis of guanosine

2.2. Glycosylation of 2-N-Acetyl-6-O-(diphenylcarbamoyl)guanine with 1,2,3,4,6-Penta-O-acetyl-α-D-glucopyranose

D-Glucose was peracetylated in pyridine to give 1,2,3,4,6-penta-O-acetyl-α-D-glucopyranose (91%) as the only isolated product. Coupling of persilylated 2-N-acetyl-6-O-(diphenylcarbamoyl)guanine with 1,2,3,4,6-penta-O-acetyl-α-D-glucopyranose (6) using TMSOTf as Lewis acid gave the protected nucleoside (68%). This reaction required 5 h, and multiple minor spots were observed on TLC. Deprotection of 7 in NH$_3$/H$_2$O/MeOH at 60 °C gave 9-(β-D-glucopyranosyl)guanine as the sole product (44%) (Scheme 2). The regioisomer structure assignment was based on the correlation of $^{13}$C NMR chemical shifts (Figure 1).
Figure 1. Determination of N7 and N9 isomers by $^{13}$C NMR

Scheme 2. Synthesis of 9-(β-D-glucopyranosyl)guanine
2.3. Glycosylation of 2-N-Acetyl-6-O-(diphenylcarbamoyl)guanine with 2-Acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-α-D-glucopyranose

D-Glucosamine hydrochloride was peracetylated in pyridine to give 2-acetamido-1,3,5,6-tetra-O-acetyl-2-deoxy-α-D-glucopyranose (86%) as the only isolated product (Scheme 3). Attempts to transform 2-acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-α-D-glucopyranose into 1,3,4,6-tetra-O-acetyl-2-deoxy-2-trifluoroacetamido-α-D-glucopyranose via reported procedures\(^{33,34}\) were unsuccessful. Treatment of 2-acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-α-D-glucopyranose with HBr in glacial acetic acid (30% w/w HBr) gave 1,3,5,6-tetra-O-acetyl-2-amino-2-deoxy-D-glucopyranose hydrobromide (51%). Deprotonation with bases, however, usually gave rearrangement products via oxazoline or orthoacetate intermediates depending on the reaction conditions (Figure 2).\(^{35}\)

![Figure 2. Acetyl rearrangement in 1,3,4,6-tetra-O-acetylglucosamine](image)

Therefore, in the second route, 1,3,4,6-tetra-O-acetyl-2-deoxy-2-trifluoroacetamido-α-D-glucopyranose was prepared by formation of 2-deoxy-2-trifluoroacetamido-D-glucopyranose (41%)\(^{36}\) followed by peracetylation (34%).
Scheme 3. Preparation of 1,3,4,6-tetra-O-acetyl-2-deoxy-2-trifluoroacetamido-α-D-glucopyranose

Disappearance of persilylated 2-N-acetyl-6-O-(diphenylcarbamoyl)guanine (2) in coupling with 2-acetamido-1,3,4,6-tetra-O-acetyl-α-D-glucopyranose (13) in toluene at 80 °C using TMSOTf as catalyst was complete in 3 h, and gave multiple spots on TLC. Significant loss of the 6-O-diphenylcarbamoyl group was observed. Deprotection of the mixture in NH₃/H₂O/MeOH at 60 °C gave crude 9-(2-acetamido-2-deoxy-β-D-glucopyranosyl)guanine as the sole product (Scheme 4). The regioisomer structure was assigned based on the correlation of ¹³C NMR chemical shifts (Figure 1). Formation of the N9 nucleoside as the major product, which was separated, might be due to reversible N7 → N9 transglycosylation in favor of the more stable product in spite of the loss of the 6-O-DPC group.
To avoid removal of the 6-\(O\)-DPC group by the formed acid, peracetyl glucosamine was treated with TMSOTf in DCE to give a fused oxazoline-sugar species. TfOH was scavenged with Hünigs base in situ, and volatiles were evaporated. The residue was dissolved in toluene and treated with the persilylated Robins reagent. However, this procedure was not successful. It is known that the fused oxazoline is a poor glycosyl donor, and its activation requires strong Brønsted acids.\(^{37-39}\)

Coupling of persilylated 2-\(N\)-acetyl-6-\(O\)-(diphenylcarbamoyl)guanine with 1,3,4,6-tetra-\(O\)-acetyl-2-trifluoroacetamido-2-deoxy\(-\alpha\)-D-glucopyranose in toluene at 80 °C with TMSOTf gave multiple spots on TLC. The 2-trifluoroacetamido function did not participate well in glycosylation.

\[
\begin{align*}
\text{Oxazoline-Sugar Species} & \quad 1. \text{BSA, DCE 80 °C} \\
& \quad 2. \text{TMSOTf, toluene, 13 80 °C} \\
\end{align*}
\]

\[
\begin{align*}
\text{2} & \quad \text{18} \\
& \quad \text{19}
\end{align*}
\]

**Scheme 4. Coupling of the Robins reagent with 2-acetamido-1,3,4,6-tetra-\(O\)-acetyl-2-deoxy-\(-\alpha\)-D-glucopyranose**

Timoshchuk, et al. reported a synthesis of a 2-amino-2-deoxy-D-glucopyranuronic acid nucleoside via a fusion method after failures of methods such as Vorbrüggen’s condensation.\(^{40}\) The 6-\(O\)-DPC group can be easily removed in 90% aqueous TFA solution.\(^{41}\) The failure to observe a significant amount of the desired product from coupling of persilylated 2-\(N\)-acetyl-6-\(O\)-(diphenylcarbamoyl)guanine with 1,3,4,6-tetra-\(O\)-acetyl-2-trifluoroacetamido-2-deoxy-D-glucopyranose might be caused by the high activation energy required to form an oxocarbenium ion due to the strongly
electron withdrawing CF₃CO group. Also, the trifluoromethanesulfonic acid formed removes the 6-O-diphenylcarbamoyl function as shown in Figure 3. It was reported that N9-vinylation of 2-N-acetyl-6-O-(diphenylcarbamoyl)guanine in refluxing vinyl acetate gave “drastically decreased” regioselectivity upon the addition of a small amount of sulfuric acid.⁴²

Treatment of peracetyl-D-glucosamine (13, X = CH₃) with SnCl₄ (82% from the α anomer and 88% from the β anomer),⁴³ TMSOTf (88%)³⁹,⁴⁴ or other Lewis acids and acids⁴⁵ has been reported to give the fused 2-methyloxazoline 9 (X = CH₃). During coupling of persilylated 2-N-acetyl-6-O-(diphenylcarbamoyl)guanine with 13, the triflate salt of 9 was formed (¹H NMR). The salt acts as a proton source to remove the 6-O-diphenylcarbamoyl group. However, the formed oxazoline can be glycosidated. Such oxazolines have been used as precursors for the stereodirected synthesis of 1,2-trans-glycosides and oligosaccharides.⁴⁵,⁴⁶ The main limitations are the harsh conditions (high temperature and acidity) required for the condensations, which cause the deprotection of acid-sensitive groups. Thus reactive glycosyl acceptors are required.⁴⁸ The amide group was also a negative factor for failures of 2-N-acetylglucosamine to function as a glycosyl acceptor at its 4-hyroxyl group.⁴⁷,⁴⁸ Alternative protecting groups were employed for its glycosylation.⁴⁷-⁴⁹
Figure 3. Acid-catalyzed removal of 6-O-DPC

(Trifluoromethyl)oxazoline derivatives have been used as glycosyl donors, and were prepared by cyclization with 2,6-lutidine in good yields.\textsuperscript{50a-c} The lower stability of this fused oxazoline is well documented. An oxazoline (18\%) was observed in the reaction of 3,4,6-tri-\textit{O}-acetyl-2-deoxy-2-trifluoroacetamido-D-glucose under Koenigs-Knorr conditions (Ag\textsubscript{2}CO\textsubscript{3}/BnOH), with a glycal formed as the major product (62\%).\textsuperscript{50d} Similar results were obtained under modified conditions (Ag\textsubscript{2}O/MeCN/H\textsubscript{2}O) to give the (trifluoromethyl)oxazoline (12\%) among other products.\textsuperscript{50c} An aziridine instead of an oxazoline was observed as an intermediate during the construction of thioglycosides,
which suggested the lower stability of the (trifluoromethyl)oxazoline. In our coupling of persilylated 2-N-acetyl-6-O-(diphenylcarbamoyl)guanine with 1,3,4,6-tetra-O-acetyl-2-deoxy-2-trifluoroacetamido-D-glucopyranose, an oxazoline might be formed as a minor species. The silylated monocyclic oxocarbenium ion and/or olefin might also be formed due to the strong electron withdrawing effect of the CF₃CO group (Figure 3). The silylated oxocarbenium ion is not an effective glycosyl donor due to its steric hindrance, and the high energy content of these derivatives decrease the reversibility of N7 → N9 transglycosylation. Thus the N7 nucleoside formed would be trapped.

2.4. Glycosylation of 2-N-Acetyl-6-O-(diphenylcarbamoyl)guanine with 1,3,4,6-Tetra-O-acetyl-2-deoxy-2-phthalimido-D-glucopyranose

In order to eliminate the oxazoline-formation effect of an amide group, the 2-amino function could be masked as a 2-azido or 2-phthalimido group. Because an azide is not a good participating group, an anomeric mixture of glycosylation products is formed. Phthalimido as a participating group strongly delocalizes the formed oxocarbenium ion, and also obstructs the formation of α-glycosides by steric hindrance and neighboring group participation. Lewis acids such as SnCl₄, BF₃·Et₂O, TMSOTf, etc. were used to activate 1-O-acetalsugars to give the 1,2-trans-2-deoxy-2-phthalimidoglycosides.

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-phthalimido-D-glucopyranose was prepared according to the reported procedure. D-Glucosamine hydrochloride was deprotonated with MeONa/MeOH, and treated with powdered phthalic anhydride and Et₃N to give the triethylammonium salt of 2-(2-carboxybenzamido)-2-deoxy-D-glucopyranose (13%). The low yield was caused by the inefficient deprotonation of D-glucosamine hydrochloride.
The salt was peracetylated in pyridine to give 1,3,4,6-tetra-\(O\)-acetyl-2-deoxy-2-phthalimido-\(D\)-glucose (40%) (Scheme 5).

**Scheme 5. Preparation of 1,3,4,6-tetra-\(O\)-acetyl-2-deoxy-2-phthalimido-\(D\)-glucose and its activation**

Coupling of persilylated 2-\(N\)-acetyl-6-\(O\)-(diphenylcarbamoyl)guanine with 1,3,4,6-tetra-\(O\)-acetyl-2-deoxy-2-phthalimido-\(D\)-glucopyranose in toluene at 80 °C using TMSOTf gave multiple spots on TLC, with the desired product as the major component. The reaction remained incomplete after 6 h. Deprotection of the mixture in \(NH_3/MeOH\) (saturated at -14 °C) at 65 °C gave the crude product in low yield contaminated with a byproduct that was difficult to remove.

Whitfield et al. studied neighboring group assistance in glycosylation reactions using a dynamic density functional theory (DFT). They proposed that the 2,6-di-\(O\)-acetyl-3,4-\(O\)-isopropylidene-\(D\)-galactopyranosyl cation existed in two conformers characterized as \(2\,S_O\) and \(B_{2,5}\). The \(2\,S_O\) conformer has the 2-\(O\)-acetyl group equatorial with the carbonyl \(syn\) to H2 and is populated by monocyclic oxocarbenium ions; the \(B_{2,5}\) conformer has O2 axial and allows the carbonyl to rotate and close the five-membered ring to form the bicyclic dioxocarbenium ion (Figure 4). The bicyclic dioxocarbenium ion is more stable than the monocyclic oxocarbenium ion (14.1 kcal·mol\(^{-1}\)). Crich et al. observed the bridging 2-phenyl-1,3-dioxocarbenium ion based on \(^{13}C\) NMR. The
lowering of the energy of the oxocarbenium ion by a phthalimido group should benefit the N7 → N9 transglycosylation.

**Figure 4. Stabilization of an oxocarbenium ion by the anchimeric effect**

The results of coupling 2-N-acetyl-6-O-(diphenylcarbamoyl)guanine with ribofuranose and glucopyranose derivatives showed that the glycosylation of ribofuranose is much faster than that of glucopyranose. The difference in reaction rates is determined by the different activation energies, and the energy barrier for the formation of the bicyclic dioxocarbenium ion is involved (Figures 5,6). It is well known that hydrolysis of the O-glycosidic bond of furanosides is about two powers of ten faster than that of pyranosides.37 Fast reactions allow less chance for the loss of the 6-O-diphenylcarbamoyl function caused by adventitious H₂O.

To shorten the reaction time, the anomic site needed to be further activated. A standard method is to transform the sugar derivative into a pyranosyl chloride or bromide (the Koenigs-Knorr method).63 But, activation for glycosylation requires halophilic promoters, usually heavy-metal salts, which result in irreversible glycosyl transfer to the receptor. Handlon and Fraser-Reid64 developed a method to prepare β-linked N-glycopeptides from pentenyl glycosides with the 2-amino group protected as a phthalimido function. Activation of the glycosyl donor as a trichloroacetimidate has been widely utilized in syntheses of oligosaccharides65-68 and of natural products.69,70 The combination of a 2-phthalimido group and 1-O-trichloroacetimidate activation has
numerous applications in syntheses of oligosaccharides.\textsuperscript{39, 71, 72} The trichloroacetimidate group can be activated with mild Lewis acids such as BF\textsubscript{3}·Et\textsubscript{2}O,\textsuperscript{71} TMSOTf,\textsuperscript{39} etc. Thus, this activated sugar intermediate was considered suitable to enhance the rate of glycosylation.

\includegraphics[width=\textwidth]{figure5.png}

\textbf{Figure 5. Mechanism of glycosylation under Lewis acid conditions}

\includegraphics[width=\textwidth]{figure6.png}

\textbf{Figure 6. Approximate Relative Activation energies for the formation of oxocarbenium cations}
2.5. Glycosylation of 2-N-Acetyl-6-O-(diphenylcarbamoyl)guanine with 3,4,6-Tri-O-acetyl-1-O-trichloroacetamidoyl-2-deoxy-2-phthalimido-β-D-glucopyranose

It was reported that both the α- and β-trichloroacetimidates can be prepared in pure form and in high yield with different bases. The β-trichloroacetimidate was formed preferentially, or even exclusively from an α/β-pyranose mixture, in a rapid and reversible addition reaction. The β-anomer anomerizes to the thermodynamically more stable α-trichloroacetimidate. The reaction also gives β-glycosides via an S_N1 mechanism with anchimeric assistance.

The β-trichloroacetimidate was prepared by a reported procedure. 1,3,4,6-Tetra-O-acetyl-2-deoxy-2-phthalimido-D-glucopyranose was treated with hydrazine acetate in dried DMF to give 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-D-glucopyranose (70%). This hemiacetal was deprotonated with DBU, and reacted with trichloroacetonitrile to give the β-trichloroacetimidate (78%) (Scheme 5).

Persilylated 2 disappeared from the coupling reaction with this trichloroacetimidate in ~2 h, but multiple spots were observed on TLC. The time was then shortened to 20-30 min to give a clean coupling product with an acceptable yield (52–54%). Deprotection in methanolic NH_3 at -10 °C–ambient temperature gave clean cleavage of the 6-carbamoyl function and acetyl groups, and a partially deprotected product precipitated (31%, not optimized) (Scheme 6, Figure 7). Deprotection at 65 °C gave a byproduct that was difficult to remove.
Scheme 6. Coupling of the Robins reagent with 3,4,6-tri-O-acetyl-2-deoxy-1-O-trichloroacetamidoyl-2-phthalimido-β-D-glucopyranose

3. Conclusions

Generally, the effectiveness of the coupling of hexopyranose derivatives (glucose and glucosamine: brown to black reaction mixtures and solid precipitations) is poorer than with pentofuranose derivatives (ribose: clear, light-colored solutions). The different reaction rates might be caused by the different activation energies required to form oxocarbenium ions as suggested by hydrolysis rates for O-glycosidic bonds. It is well known that hydrolysis of furanosides is about two powers of ten faster than that of pyranosides. Migration of hypoxanthine from ribose to glucose when 2’,3’-O-isopropylideneinosine was heated with 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide is in harmony with the higher energy of a pyranose oxocarbenium ion, which decreases the reversibility of glycosylation and protects the pyranosides from donating the heterocyclic base.
Figure 7. $^1$H NMR spectrum of compound 24

The regioselective N9 alkylation and glycosylation of 2-N-acetyl-6-O-(diphenylcarbamoyl)guanine catalyzed by Lewis acids is governed by several factors, e.g., the size and reactivity of electrophiles and reaction conditions. When the oxocarbenium ion is very unstable, the glycosylation is irreversible (e.g., 3,4,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-D-glucose). With an increase in stability of the reaction intermediate, reversibility of the reaction increases, and the reaction is under increased thermodynamic control. This conclusion is well supported by the observed effect of 2-O-protecting groups on the regioselectivity. A bulky 6-functional group on guanine may effectively exclude attack at N7 at the kinetic level, and also shift the equilibrium of transglycosylation to N9 nucleoside at the thermodynamic level. Reaction temperature is another important factor. An increase in reaction temperature increases the
reversibility of transglycosylation.\textsuperscript{4} It appears that the 6-O-DPC group cannot exclude attack at N7 by very reactive electrophiles.

![Potential energy diagram for Lewis acid catalyzed glycosylation](image)

**Figure 8. Potential energy diagram for Lewis acid catalyzed glycosylation**

In summary, regioselective N9 glycosylation of guanine with glucosamine has been accomplished by protecting the 2-amino group as a phthalimido function, which stabilizes the oxocarbenium cation. The activation energy was lowered further by introduction of an anomeric trichloroacetimidate leaving group (Figure 8).

4. Experimental Section

2-N-Acetyl-6-O-(diphenylcarbamoyl)guanine (2)

Compound 2 was prepared by a reported method.\textsuperscript{5,23} 2-N,9-Diacetylguanine (5.4 g, 87\%): \textsuperscript{1}H NMR (300 MHz, DMSO-\textit{d}_6) \( \delta \) 2.20 (s, 3H), 2.80 (s, 3H), 8.44 (s, 1H).
Compound 2 (6.1 g, 81%): \(^1H\) NMR (500 MHz, DMSO-\(d_6\)) \(\delta\) 2.15 (s, 3H), 7.29–7.48 (m, 10H), 8.42 (s, 1H), 10.59 (s, 1H), 13.54 (s, 1H); \(^13\)C NMR (125 MHz, DMSO-\(d_6\)) \(\delta\)
169.2, 156.6, 155.4, 152.6, 150.9, 144.7, 142.4, 130.1, 127.9, 120.5, 25.1; LRMS (FAB) \(m/z\) 389 (MH\(^+\) \([C_{20}H_{17}N_6O_3]\) = 389).

**Guanosine (4)**

BSA (1.0 mL, 814 mg, 4.0 mmol) was added to a stirred suspension of 2 (776 mg, 2.0 mmol) in dried DCE (20 mL), and stirring was continued under \(N_2\) at 80 °C for 30 min. The clear solution was evaporated, and the residue was dried under high vacuum for 15 min and dissolved in dried toluene (10 mL). TMSOTf (0.64 mL, 786 mg, 3.5 mmol) and a solution of 1-O-acetyl-2,3,5-tri-O-benzoyl-\(\beta\)-D-ribofuranose (1.24 g, 2.5 mmol) in dried toluene (10 mL) were added. The solution was stirred at 80 °C for 3 h and volatiles were evaporated in vacuo. The residue was dissolved in EtOAc, and the solution was washed (NaHCO\(_3\)/H\(_2\)O, 2 x 100 mL; brine 2 x 100 mL) and dried (Na\(_2\)SO\(_4\)). Volatiles were evaporated, and the residue was chromatographed (CH\(_2\)Cl\(_2\) → CH\(_2\)Cl\(_2\)/MeOH 1:180 → 1:90) to give the protected nucleoside 3 (1.6 g, 96%) as a white foam: LRMS (FAB) \(m/z\) 833 (MH\(^+\) \([C_{46}H_{37}N_6O_{10}]\) = 833), 855 (MNa\(^+\) \([C_{46}H_{36}N_6O_{10}Na]\) = 855).

NH\(_3\)/H\(_2\)O (29%, 40 mL) was added to a solution of 3 (1.18 g, 1.42 mmol) in MeOH (40 mL), and the solution was stirred in a sealed flask at 60 °C for 72 h. Volatiles were evaporated in vacuo, and the residue was recrystallized from H\(_2\)O to give guanosine 4 (280 mg, 70%).

**1,2,3,4,6-Penta-O-acetyl-\(\alpha\)-D-glucopyranose (6)**

D-Glucose (2.0 g, 11.1 mmol) was added to a stirred, ice-cold solution of Ac\(_2\)O (10 mL) and pyridine (14 mL). After the starting material had dissolved, the solution was
allowed to gradually warm to ambient temperature, and was stirred overnight. The reaction mixture was poured into ice-water (40 mL), and was stirred for 1 h. The resulting precipitate was collected by filtration, washed twice with ice-water, and dried to give compound 6 (3.96 g, 91%): $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 2.02 (s, 3H), 2.03 (s, 3H), 2.05 (s, 3H), 2.10 (s, 3H), 2.18 (s, 3H), 4.08–4.14 (m, 2H), 4.27 (dd, $J$ = 6.5, 4.5 Hz, 1H), 5.09–5.18 (m, 2H), 5.48 (t, $J$ = 10.0 Hz, 1H), 6.33(d, $J$ = 3.6 Hz, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 170.8, 170.4, 169.9, 169.7, 169.0, 89.3, 70.1, 69.4, 68.1, 61.7, 21.1, 20.9, 20.9, 20.8, 20.7; LRMS (EI) m/z 331 (M – 59 [C$_{14}$H$_{19}$O$_9$] = 331), in agreement with published data.$^{79,80}$

9-(β-D-Glucopyranosyl)guanine (8)

BSA (1.0 mL, 814 mg, 4 mmol) was added to a stirred suspension of 2 (776 mg, 2 mmol) in dried DCE (20 mL), and stirring was continued under N$_2$ at 80 °C for 30 min. The clear solution was evaporated, the residue was dried under high vacuum for 1.5 h and dissolved in dried toluene (10 mL). TMSOTf (0.64 mL, 772 mg, 3.6 mmol) and a solution of 6 (1.0 g, 2.6 mmol) in dried toluene (10 mL) were added, and the solution was stirred at 80 °C for 5 h. Volatiles were evaporated in vacuo, and the residue was dissolved in EtOAc. The solution was washed (NaHCO$_3$/H$_2$O, 3 x 100 mL) and dried (Na$_2$SO$_4$). Volatiles were evaporated, and the residue was chromatographed (Et$_2$O → Me$_2$CO/Et$_2$O, 1:4) to give the protected nucleoside 7 (970 mg, 68%) as a white foam.

$\text{NH}_3$/H$_2$O (29%, 20 mL) was added to a solution of 7 (326 mg, 0.45 mmol) in MeOH (20 mL), and the solution was stirred at 60 °C overnight in a sealed flask.

Volatiles were evaporated, and the solid was washed with CHCl$_3$ to give 8 (64 mg, 44%): UV (MeOH) max 254 nm ($\varepsilon$ 13 600), min 222 nm ($\varepsilon$ 2900); $^1$H NMR (500 MHz, DMSO-
$d_6$ δ 3.17–3.31 (m, 3H), 3.39–3.44 (m, 1H), 3.66–3.69 (m, 1H), 3.77–3.82 (m, 1H), 4.57 (t, $J = 6.0$ Hz, 1H), 5.08 (d, $J = 5.4$ Hz, 1H), 5.14 (d, $J = 9.2$ Hz, 1H), 5.22 (d, $J = 4.3$ Hz, 1H), 5.32 (d, $J = 5.4$ Hz, 1H), 6.47 (br s, 2H), 7.84 (s, 1H), 10.38 (s, 1H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 157.4, 154.3, 152.3, 136.4, 117.0, 82.8, 80.8, 78.1, 72.0, 70.4, 61.6; HRMS $m/z$ 314.1109 (MH$^+$ [C$_{11}$H$_{16}$N$_5$O$_6$] = 314.1101).

**2-Acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-α-D-glucopyranose (13)**

Compound 13 was prepared by a reported method.$^{81}$ Yield (6.76 g, 86%): $^1$H NMR (500 MHz, CDCl$_3$) δ 1.94 (s, 3H), 2.05 (s, 3H), 2.06 (s, 3H), 2.09 (s, 3H), 2.20 (s, 3H), 3.98–4.01 (m, 1H), 4.07 (dd, $J = 12.4$, 2.4 Hz, 1H), 4.25 (dd, $J = 12.4$, 4.0 Hz, 1H), 4.49 (dt, $J = 6.1$, 3.7 Hz, 1H), 5.19–5.26 (m, 1H), 5.54 (d, $J = 8.9$ Hz, 1H), 6.18 (d, $J = 3.7$ Hz, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 172.0, 170.9, 170.1, 169.3, 168.8, 90.9, 70.9, 70.0, 67.7, 61.8, 51.3, 23.3, 21.2, 20.94, 20.91, 20.8; LRMS (CI) $m/z$ 390, 330 (MH$^+$ [C$_{16}$H$_{24}$NO$_{10}$] = 390, M – 59 [C$_{14}$H$_{20}$NO$_{8}$] = 330), in agreement with published data.$^{81,82}$

**1,3,4,6-Tetra-O-acetyl-2-amino-2-deoxy-α-D-glucopyranose hydrobromide (14)**

Compound 14 was prepared by a reported method.$^{33}$ Yield (2.8 g, 51%): $^1$H NMR (500 MHz, DMSO-$d_6$) δ 1.99 (s, 3H), 2.00 (s, 3H), 2.05 (s, 3H), 2.20 (s, 3H), 3.92–4.01 (m, 1H), 4.00 (d, $J = 11.9$ Hz, 1H), 4.15–4.22 (m, 2H), 5.01 (t, $J = 9.6$ Hz, 1H), 5.25 (t, $J = 10.0$ Hz, 1H), 6.18 (d, $J = 3.3$ Hz, 1H), 8.48 (s, 3H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 170.0, 169.2, 168.7, 88.2, 69.0, 68.8, 67.3, 61.0, 50.2, 20.8, 20.5, 20.3; HRMS $m/z$ 348.1283 (M – Br [C$_{14}$H$_{22}$NO$_9$] = 348.1295), 288.1070 (M – H – 59 [ C$_{12}$H$_{18}$NO$_7$] = 288.1083); LRMS $m/z$ 348, 306, 288.
Rearrangement of the acetyl group from O1 to N2 in compound 10

Compound 14 (0.1 g, 0.23 mmol) in MeONa/H₂O [prepared by addition of MeONa/H₂O (40 mg, 0.74 mmol) to H₂O (10 mL)] or AcONa/H₂O solution was stirred for 1 h. The mixture was extracted with CHCl₃ (3 x), and volatiles were evaporated to give the crude product 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranose (15): HRMS m/z 370.1127 (MNa⁺ [C₁₄H₂₁NO₉Na] = 370.1114), 352.1015 (MNa - OH [C₁₄H₁₉NO₈Na] = 352.1008); The product formed depended on the reaction conditions.

Methyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranoside was also formed: ¹H NMR (300 MHz, CDCl₃) δ 1.96 (s, 3H), 2.03 (s, 3H), 2.04 (s, 3H), 2.10 (s, 3H), 3.51 (s, 3H), 3.68–3.77 (m, 1H), 3.87 (dt, J = 8.5, 10.5 Hz, 1H), 4.15 (dd, J = 2.6, 13.0 Hz, 1H), 4.29 (dd, J = 2.6, 13.0 Hz, 1H), 4.60 (d, J = 8.4 Hz, 1H), 5.08 (t, J = 9.7 Hz, 1H), 5.28 (t, J = 9.5 Hz, 1H), 5.76 (d, J = 8.8 Hz, 1H); ¹³C NMR (125 MHz, CDCl₃) δ 171.0, 170.9, 170.4, 169.5, 101.7, 72.5, 71.9, 68.7, 62.2, 56.9, 54.6, 23.5, 20.9, 20.8, 20.7; LRMS (FAB) m/z 362, 330 (MH⁺ [C₁₅H₂₃NO₉]= 362, M – 31 [C₁₄H₂₀NO₈]= 330).

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-trifluoroacetamido-D-glucopyranose (17)

Compound 16 was prepared by a reported method.³⁶ Yield (2.6 g, 41%): LRMS m/z 276 (MH⁺ [C₈H₁₃F₃NO₆]= 276), 258 (M – 17 [C₈H₁₁F₃NO₅]= 258).

Ac₂O (15 mL) was added to a solution of 16 (2.57 g, 9.0 mmol) in pyridine (15 mL) at 0 °C, and the mixture was stirred overnight. H₂O (10 mL) was added, and the mixture was extracted with CH₂Cl₂. The organic layer was washed [HCl/H₂O (1 N, 50 mL), H₂O (50 mL), NaHCO₃/H₂O (2 x 50 mL), and brine (2 x 50 mL)] and dried (MgSO₄). Volatiles were evaporated in vacuo, and the residue was washed EtOAc/hexanes (1:1), and crystallized from EtOAc/hexanes to give the anomeric mixture
(17) (α/β, 3.8:1; 1.41 g, 34%). α: 1H NMR (500 MHz, CDCl3) δ 2.06 (s, 3H), 2.07 (s, 3H), 2.10 (s, 3H), 2.21 (s, 3H), 4.01–4.05 (m, 1H), 4.08 (dd, J = 12.6, 2.2 Hz, 1H), 4.29 (dd, J = 12.9, 4.4 Hz, 1H), 4.41–4.45 (m, 1H), 5.22–5.33 (m, 2H), 6.26 (d, J = 3.6 Hz, 1H), 6.44 (d, J = 8.8 Hz, 1H); β: δ 2.05 (s, 3H), 2.06 (s, 3H), 2.10 (s, 3H), 2.12 (s, 3H), 3.82–3.85 (m, 1H), 4.14 (dd, J = 12.5, 2.2 Hz, 1H), 4.29 (dd, J = 12.9, 4.4 Hz, 1H), 5.15–5.22 (m, 1H), 5.22–5.33 (m, 2H), 5.75 (d, J = 8.5 Hz, 1H), 6.49 (d, J = 9.4 Hz, 1H);
HRMS m/z 466.0955 (MNa+ [C16H20F3NO10Na] = 466.0937).

9-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)guanine (19)

BSA (0.50 mL, 407 mg, 2.0 mmol) was added to a stirred suspension of 2 (390 mg, 1 mmol) in dried DCE (10 mL), and stirring was continued under N2 at 80 °C for 40 min. The clear solution was evaporated, and the residue was dried under high vacuum overnight, and dissolved in dried toluene (5 mL). TMSOTf (0.32 mL, 386 mg, 1.8 mmol) and a solution of 13 (0.47 g, 1.2 mmol) in dried toluene (15 mL, low solubility) were added. The suspension was stirred at 80 °C under N2 for 3.3 h. A black sticky layer on the flask inner wall was observed, and TLC showed several spots. Volatiles were evaporated in vacuo. The residue was dissolved in EtOAc, and the solution was washed (NaHCO3/H2O, 3 x 50 mL) and dried (Na2SO4). Volatiles were evaporated in vacuo, and the residue was chromatographed (CH2Cl2 → CH2Cl2/MeOH, 1:180 → 1:90) to give three components. The first two eluted were dissolved in NH3/H2O/MeOH (20 mL/20 mL) in a sealed flask and stirred at 60 °C for 20 h. Volatiles were evaporated, and the residue was washed well with CHCl3 to give a crude product (275 mg): 1H NMR (300 MHz, DMSO-d6) δ 1.63 (s, 3H), 3.15–3.67 (m, 5H), 4.14 (q, J = 10 Hz, 1H), 5.31 (d, J = 10.3 Hz, 1H), 6.56 (br, 2H), 7.65 (s, 1H), 7.92 (d, J = 8.3 Hz, 1H) (OH peaks very
broadened); $^{13}$C NMR (75 MHz, DMSO-$d_6$) δ 169.9, 157.5, 154.5, 152.2, 136.0, 116.8, 80.9, 80.6, 75.1, 70.7, 61.4, 53.9, 23.5.

**The 2-Methyloxazolinium Triflate Salt (9) Formed by Cyclization of 13 with TMSOTf**

To a stirred solution of 13 (58.5 mg, 0.15 mmol) in toluene (5 mL) at 80 °C under N$_2$ was added TMSOTf (0.08 mL, 94.5 mg, 0.40 mmol). The solution was stirred for 1 h. Volatiles were evaporated in vacuo to give a residue: $^1$H NMR (500 MHz, CDCl$_3$) δ 2.11 (s, 6H), 2.12 (s, 3H), 2.61 (s, 3H), 3.91–3.95 (m, 1H), 4.30 (d, $J$ = 4.6 Hz, 2H), 4.76 (d, $J$ = 7.5 Hz, 1H), 5.06–5.08 (m, 1H), 5.30 (t, $J$ = 3.0 Hz, 1H), 6.74 (d, $J$ = 8.2 Hz, 1H), 12.03 (s, 1H); $^{19}$F NMR (282 MHz, CDCl$_3$) δ -79.8.

This reaction was repeated in DCE to give the same compound.

**1,3,4,6-Tetra-O-acetyl-2-deoxy-2-phthalimido-D-glucopyranose (21)**

Compound 21 was prepared by a method reported by Lemieux.$^{58}$ Recrystallization from EtOAc gave the β-anomer: $^1$H NMR (500 MHz, CDCl$_3$) δ 1.87 (s, 3H), 2.01 (s, 3H), 2.05 (s, 3H), 2.12 (s, 3H), 4.01–4.05 (m, 1H), 4.16 (dd, $J$ = 10.3, 2.0 Hz, 1H), 4.37 (dd, $J$ = 12.6, 4.3 Hz, 1H), 4.48 (dd, $J$ = 10.3, 8.8 Hz, 1H), 5.22 (t, $J$ = 9.6 Hz, 1H), 5.89 (dd, $J$ = 10.7, 9.2 Hz, 1H), 6.52 (d, $J$ = 8.9 Hz, 1H), 7.75–7.78 (m, 2H), 7.86–7.88 (m, 2H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 170.7, 170.02, 169.5, 168.6, 167.4, 134.5, 131.3, 123.8, 89.8, 72.7, 70.5, 68.3, 61.6, 53.5, 20.78, 20.76, 20.6, 20.4; HRMS $m/z$ 500.1186 (MNa$^+$ [C$_{22}$H$_{23}$NO$_{11}$Na] = 500.1169), in agreement with published data.$^{83}$
3,4,6-Tri-O-acetyl-2-deoxy-1-O-trichloroacetimidoyl-2-phthalimido-β-D-glucopyranose (22)

Compound 22 was prepared by a reported method.\textsuperscript{83,84} Yield for 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose (1.0 g, 70%): \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) \(\delta\) 1.89 (s, 3H), 2.06 (s, 3H), 2.14 (s, 3H), 3.23 (d, \(J = 7.3\) Hz, 1H), 3.94–3.97 (m, 1H), 4.20–4.33 (m, 3H), 5.20 (t, \(J = 9.6\) Hz, 1H), 5.65 (t, \(J = 7.8\) Hz, 1H), 5.88 (t, \(J = 8.8\) Hz, 1H), 7.75–7.77 (m, 2H), 7.87–7.88 (m, 2H); \textsuperscript{1}H NMR (500 MHz, DMSO-\textsubscript{d}6) \(\delta\) 1.79 (s, 3H), 2.00 (s, 3H), 2.05 (s, 3H), 3.98–4.03 (m, 2H), 4.08 (dd, \(J = 12.2, 2.1\) Hz, 1H), 4.19 (dd, \(J = 12.1, 4.8\) Hz, 1H), 4.97 (t, \(J = 9.6\) Hz, 1H), 5.45 (dd, \(J = 8.3, 6.1\) Hz, 1H), 5.64 (t, \(J = 10.0\) Hz, 1H), 7.52 (d, \(J = 6.1\) Hz, 1H), 7.89–7.93 (m, 4H); HRMS m/z 458.1062 (MNa\textsuperscript{+}[C\textsubscript{20}H\textsubscript{21}NO\textsubscript{10}Na] = 458.1063). Yield for 22 (3.6 g, 62%): \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) \(\delta\) 1.90 (s, 3H), 2.05 (s, 3H), 2.13 (s, 3H), 4.05–4.09 (m, 1H), 4.21 (dd, \(J = 12.5, 2.2\) Hz, 1H), 4.39 (dd, \(J = 12.3, 4.4\) Hz, 1H), 4.64 (dd, \(J = 10.7, 9.2\) Hz, 1H), 5.28 (t, \(J = 9.7\) Hz, 1H), 5.92 (dd, \(J = 10.7, 9.3\) Hz, 1H), 6.63 (d, \(J = 8.8\) Hz, 1H), 7.72–7.74 (m, 2H), 7.83–7.85 (m, 2H), 8.66 (s, 1 H); \textsuperscript{13}C NMR (125 MHz, CDCl\textsubscript{3}) \(\delta\) 170.9, 170.3, 169.7, 167.6, 160.8, 134.7, 131.5, 123.9, 93.8, 90.4, 73.1, 70.7, 68.7, 61.8, 53.8, 21.0, 20.9, 20.7; HRMS m/z 601.0154 (MNa\textsuperscript{+}[C\textsubscript{22}H\textsubscript{21}Cl\textsubscript{3}N\textsubscript{2}O\textsubscript{10}Na] = 601.0159), in agreement with published data.\textsuperscript{83,84} Further elution of the column (MeOH/CH\textsubscript{2}Cl\textsubscript{2} 1:30) gave a mixture (1.9 g, including 16% of 22).

9-[2-(2-Carbamoylbenzamido)-2-deoxy-β-D-glucopyranosyl]guanine (24)

BSA (0.50 mL, 407 mg, 2.0 mmol) was added to a stirred suspension of 2 (388 mg, 1 mmol) in dried DCE (10 mL), and stirring was continued under N\textsubscript{2} at 80 °C for 30 min. The clear solution was evaporated, and the residue was dried under high vacuum for
15 min, and dissolved in dried toluene (5 mL). TMSOTf (0.32 mL, 386 mg, 1.8 mmol) and a solution of 22 (1.42 g, 1.5 mmol) in dried toluene (5 mL) were added. The solution was stirred at 80 °C for 30 min, and volatiles were evaporated in vacuo. The residue was dissolved in EtOAc, and the solution was washed (NaHCO$_3$/H$_2$O, 3 x 50 mL) and dried (Na$_2$SO$_4$). Volatiles were evaporated in vacuo, and the residue was chromatographed (CH$_2$Cl$_2$ → CH$_2$Cl$_2$/MeOH, 1:180 → 1:90) to give the fully protected nucleoside (453 mg, 54%) as a white foam: HRMS $m/z$ 828.2258 (MNa$^+$ [C$_{40}$H$_{35}$N$_7$O$_{12}$Na] = 828.2241).

A solution of this material (416 mg, 0.52 mmol) in NH$_3$/MeOH (20 mL, saturated at –10 °C) in a sealed flask was kept at ~ -12 °C for 40 h and then stirred at ambient temperature for 24 h. The precipitated solid was washed with cold MeOH to give compound 24 (73 mg, 31%): mp >250 °C; UV (MeOH) max 250 nm (ε 13 400), min 232 nm (ε 10 500); $^1$H NMR (500 MHz, DMSO-$d_6$) δ 3.28–3.33 (m, 2H), 3.48–3.50 (m, 1H), 3.69–3.75 (m, 2H), 4.29–4.33 (m, 1H), 4.65–4.67 (m, 1H), 5.00 (s, 1H), 5.28 (d, $J = 5.2$ Hz, 1H), 5.50 (d, $J = 10.3$ Hz, 1H), 6.53 (br s, 2H), 6.90 (d, $J = 7.1$ Hz, 1H), 7.40–7.45 (m, 2H), 7.50 (br s, 1H), 7.60 (d, $J = 8.1$ Hz, 1H), 7.74 (s, 1H), 7.96 (br s, 1H), 8.47(d, $J = 9.4$ Hz, 1H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 170.1, 169.7, 157.4, 154.5, 152.2, 137.6, 135.8, 134.6, 130.8, 129.8, 128.6, 127.9, 117.0, 80.8, 80.4, 75.4, 70.2, 61.4, 54.8; HRMS $m/z$ 482.1387 (MNa$^+$ [C$_{19}$H$_{21}$N$_7$O$_7$Na] = 482.1400). Anal. Calcd for C$_{19}$H$_{21}$N$_7$O$_7$·H$_2$O: C, 47.80; H, 4.86; N, 20.54. Found: C, 47.85; H, 4.75; N, 20.22.
5. References and Notes


78. Ref 4: Glycosylation of persilylated 2-N-acetylguanine with 1,2,3,4,6-penta-O-acetyl-D-glucopyranose at ambient temperature in CH$_3$CN in the presence of SnCl$_4$ gave exclusively the N7-nucleoside (kinetic control, procedure A), and gave a mixture of N9-, N7-nucleosides (2:1) in DCE/$\Delta$ in the presence of TMSOTf (partially reversible, procedure B). Glycosylation of persilylated 2-N-acetylguanine with 1,2,3,4,6-penta-O-benzoyl-D-glucopyranose gave exclusively the N7-nucleoside (kinetic control, procedure A), and a mixture of N9/N7-nucleosides (8:1) with procedure B (more readily reversible). The increase in regioselectivity results from the increased ease of reversibility of transglycosylation caused by a more stabilized oxocarbenium cation with the 2-O-benzoyl participating group.
79. Fernandez-Lorente, G.; Palomo, J. M.; Cocca, J.; Mateo, C.; Moro, P.; Terreni, M.;
Chapter 3

Syntheses of Selected 6-Heteroarylpurines and Their Regiospecific Alkylation to Give N9 Alkyl Derivatives

1. Introduction

Five-membered heteroaryl groups have been introduced at C6 of purines or C4 of pyrimidines. Gundersen et al. have studied series of 6-aryl nucleosides and acyclic analogues, and found that 9-benzyl-2-chloro-6-(2-furyl)purine had good activity against Mycobacterium tuberculosis H37-Rv (MIC, 0.78 µg/mL) and relatively low cytotoxicity. Such 6-arylpurines were prepared by Stille coupling between N9 substituted 6-chloropurines and aryl(tributyl)tin derivatives. Estep et al. reported that 6-heteroaryl-substituted purines were inactivation-modifiers of cardiac sodium channels, and five-membered aryl rings were optimal. The purine derivatives were prepared by introduction of a 6-aryl ring either by S_N2 substitution of 6-chloropurine (for 6-imidazolyl, 6-pyrazolyl, and 6-triazolyl) with an excess of the appropriate 1H-azoles, by cyclization of adenine with 1,4-dicarbonyl compounds or their equivalents in hot HOAc (for 6-pyrrolyl), or by cyclization of 6-cyanopurine with an intermediate amidoxime and triethyl orthoformate (for 6-oxadiazolyl). Alkylation via Mitsunobu reactions gave N9 alkyl derivatives whereas direct alkylation in heated DMSO (120-150 ºC) gave N3 alkyl derivatives.

Mintas et al. have synthesized series of 6-(pyrrol-1-yl)purine acyclic nucleoside analogues (Figure 1). Biological assays indicated compound 1, 2 and 3 have significant cytostatic activity against several malignant tumor cell lines. 6-(Pyrrol-1-yl)purine was
prepared from adenine and 2,5-dimethoxytetrahydrofuran in refluxing HOAc solution\textsuperscript{5,9}. The acyclic nucleoside analogues were prepared by direct alkylation under basic conditions (NaOH, or NaH in DMF).\textsuperscript{4,5,8} Alkylation occurred at both N9 and N7 with good regioselectivity for N9.\textsuperscript{5} The coplanar conformation of rings was noted with small deviations depending on the N9-alkyl groups (Figure 2); and this was disrupted by N7 alkyl groups. A pyrrole ring was recently utilized as a temporary protecting group for 2/6-amino functions of purine derivatives.\textsuperscript{10} The 2,5-dimethylpyrrole ring was introduced at C6 by heating adenine or its derivatives in neat 2,5-hexanedione at 150-160 °C for 2 days, and the amino group was regenerated in TFA/H\textsubscript{2}O.

![Figure 1. 6-(Pyrrol-1-yl)purine acyclic nucleosides](image1)

![Figure 2. Crystal structure of 9-methyl-6-(pyrrol-1-yl)purine](image2)
The triazole ring has been widely used as an intermediate to activate C6 of purines and C4 of pyrimidines for SNAr substitutions. Both 6-/4-(1,2,4-triazol-1-yl) and 6-/4-(1,2,4-triazol-4-yl) bases have been prepared and utilized in nucleoside transformations. The 6-(1,2,4-triazol-1-yl) ring was introduced directly into 6/4-oxo nucleic acid bases. Fourrey et al. reported a synthesis of 6-mercaptourine deoxynucleosides via C6 triazolylation. A 6-oxopurine derivative was treated with 1,2,4-triazole, POCl3, and Et3N in CH3CN to give the 6-(1,2,4-triazol-1-yl)purine 2'-deoxynucleoside.11 Cytosine nucleosides were prepared via ammonolysis of 4-(1,2,4-triazol-1-yl)pyrimidine intermediates.12 Pochet et al. attached DNA fragments to solid supports via alkyl amine linkages at C6 of purines or C4 of pyrimidines by replacement of 1,2,4-triazol-1-yl intermediates.13 Chu and coworkers prepared 4-azidopyrimidin-2-one nucleosides as prodrugs by replacement of a 4-(1,2,4-triazol-1-yl) ring with azide.14 The 3-nitro-1,2,4-triazol-1-yl ring was introduced as a better leaving group. Reese et al. reported successful transformations of protected guanosine and uridine derivatives to 6-(3-nitro-1,2,4-triazol-1-yl)purine and 4-(3-nitro-1,2,4-triazol-1-yl)pyrimidine nucleosides.15 These intermediates were employed for the preparation of N4-alkylated-pyrimidine and N6-alkylated-purine nucleosides.16 Kamaike et al. prepared cytosine and adenine nucleosides with the 4-/6-amino group protected as phthalimide and succinimide via nucleophilic replacement of 4-/6-(3-nitro-1,2,4-triazol-1-yl) functions.17

Recently, Robins and coworkers18,19 transformed the 6-amino group of adenine nucleosides, tubercidin, and formycin into 6-(1,2,4-triazol-4-yl) rings via cyclization with 1,2-bis[(dimethylamino)methylene]hydrazine dihydrochloride. The 6-(1,2,4-triazol-4-yl) ring was replaced by various nucleophiles. This activation strategy was applied to
prepare N6 labeled adenosines by conversion into the 6-(1,2,4-triazol-4-yl)purine nucleosides followed by nucleophilic displacement of triazole with $^{15}$N ammonia, and to introduce a linked fluorophore. Markiewicz and coworkers introduced a protected spermine moiety at C6 of 2’-deoxyadenosine via a 6-(1,2,4-triazol-4-yl)purine 2’-deoxynucleoside. Robins et al. reported the preparation of puromycin from adenosine and 7-deazapuromycin from tubercidin via 6-(1,2,4-triazol-4-yl) intermediates.

Recently, 6-(1,2,4-triazol-4-yl) was used as a directing group for regiospecific N9 alkylation of 2-aminopurine. Demeunynck et al. synthesized 2-amino-6-(1,2,4-triazol-4-yl)purine via their claimed “regiospecific” cyclization with 2,6-diaminopurine. Alkylation of this intermediate with iodomethane and propyl bromide gave N9 alkylated products.

The 6-(imidazol-1-yl) group has also been introduced into purine derivatives. The 6-(imidazol-1-yl)purine nucleosides were prepared by a modified Appel method via an oxophosphonium ion or by replacement of 6-Cl and 6-Br groups. Robins and coworkers have reported the preparation of 6-(imidazol-1-yl)purine nucleosides by treatment of protected inosines or guanosines with imidazole, I$_2$, Ph$_3$P and DIEPA in toluene at 95 °C, and found that the 6-(imidazol-1-yl) group was displaced by various nucleophiles (N,O,S).

Previous studies noted that alkylation of 6-(pyrrol-1-yl)purine gave mixtures with high regioselectivity for N9 isomers. High regioselectivity for N9 alkylation with propylene carbonate in refluxing DMF was observed either with a catalytic amount (3.8 mol%) of NaOH (N9/N7, 88.5:3.3) or with the sodium salt (N9/N7, 64.2:1.6). Regiospecific N9 alkylation of 2-amino-6-(1,2,4-triazol-4-yl)purine with sodium hydride...
and alkyl iodides at ambient temperature was reported. The difference in degree of the directing effects of these two functional groups might be caused by reaction conditions and/or electronic effects. Alkylation of 6-(imidazol-1-yl)purines should be highly regioselective, and such studies could disclose effects of 6-heteroaryl functional groups. 9-Alkyl-6-(imidazol-1-yl)purines are a new series of derivatives. The 6-(imidazol-1-yl) group on a purine ring can be replaced by nucleophiles via $S_{N}Ar$ reactions to give other series of analogues. We have studied alkylation of both 6-(imidazol-1-yl)purines and 6-(1,2,4-triazol-4-yl)purines.

2. Results and Discussion

2.1. Syntheses of Selected 6-Heteroarylpurines

2.1.1. 6-(1,2,4-Triazol-4-yl)purine

6-(1,2,4-Triazol-4-yl)purines were prepared by the method analogous to a reported procedure (Scheme 1). $N,N'$-Dimethylformamide azine dihydrochloride was prepared by treatment of $N,N'$-diformylhydrazine with thionyl chloride in DMF (83%). The azine hydrochloride and adenine were refluxed in DMF to give 6-(1,2,4-triazol-4-yl)purine (95%). However, the analogous cyclization with 2,6-diaminopurine was not regiospecific as claimed. Heating 2,6-diaminopurine and $N,N'$-dimethylformamide azine dihydrochloride in DMF gave at least two products (TLC). The unidentified byproducts were observed in varying amounts depending on the reaction conditions (Scheme 2). Suspension of these mixtures in MeOH and filtration gave almost pure 2-amino-6-(1,2,4-triazol-4-yl)purine (72%).
Scheme 1. Synthesis of 6-(1,2,4-triazol-4-yl)purines

2.1.2. 6-(Imidazol-1-yl)purine

6-(Imidazol-1-yl)purine was prepared from 2',3',5'-tri-O-acetylinosine by replacement of the 6-oxo function with imidazole\textsuperscript{25} followed by acidic deglycosylation (Scheme 2), or by replacement of the 6-oxo group of 9-tritylhypoxanthine with imidazole followed by acidic detritylation (Scheme 3).

Inosine was acetylated in Ac\textsubscript{2}O/pyridine to give 2',3',5'-tri-O-acetylinosine (95%), which was then transformed into 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-6-(imidazol-1-yl)purine under the modified Appel conditions.\textsuperscript{25} The crude product was subsequently deglycosylated (AcCl/HOAc) to give 6-(imidazol-1-yl)purine·HCl in good yield (80% for 2 steps).
Scheme 2. Synthesis of 6-(imidazol-1-yl)purine (Method 1)

Synthesis of the substituted purine bases required complete deglycosylation without removal of the 6-(imidazol-1-yl) group or ring opening of the purine. Several deglycosylation conditions were tried, as shown below. Mechanisms of acidic hydrolysis or solvolysis of purine nucleosides have been thoroughly studied.\textsuperscript{28-35} It was found to proceed by an A-1 mechanism, i.e., a preequilibrium protonation of purine followed by rate-limiting cleavage of the glycosyl bond. However, there are not many examples of preparation of purine bases via deglycosylation.\textsuperscript{36,37} Fuji et al. prepared adenine-2-$d$ (77\%) by glycosyl hydrolysis of adenosine-2-$d$ in refluxing 0.5 N aqueous HCl, and 1-ethylhypoxanthine from 1-ethylinosine.\textsuperscript{36} Therefore, deglycosylation in aqueous HCl of various concentrations was studied. Deglycosylation in 0.112 N aqueous HCl at 70 °C was incomplete after 9 h. This reaction gave multiple products including 6-(imidazol-1-yl)purine and deacetylated nucleosides. Lower temperatures (65 °C) gave the
deacetylated nucleoside as the only product after 40 h. Increased acid concentrations (1 N HCl) at elevated temperatures (85 °C) caused purine ring opening.

Robins and Robins\textsuperscript{37} reported complete acetolysis of the glycosyl bond of peracetylated 2’-dAdo in HOAc/\textsubscript{Ac2}O (4:1) at 100 °C. Deglycosylations in HCl/HOAc solutions were then evaluated. HOAc/TMScI (10 molar equivalents, 70 °C, overnight) and HOAc/AcCl (10 molar equivalents, 65 °C, overnight) gave complete deglycosylation with minor removal of the 6-(imidazol-1-yl) group. Lower concentrations of HCl (0.14 N, 5.8 molar equivalents, 65 °C, overnight) gave complete deglycosylation with only traces of hypoxanthine. The mixture was dissolved in 0.05 N NaOH/H\textsubscript{2}O, and the product was precipitated by addition of CO\textsubscript{2}. The compound is stable in NaOH/H\textsubscript{2}O (0.5 N). It dissolves to give a clear solution, and then precipitates as needle-like crystals of the sodium salt.

The same compound was also prepared from 9-tritylhypoxanthine. Hypoxanthine was tritylated according to the reported procedure for guanine.\textsuperscript{38} Persilylated hypoxanthine was treated with trityl chloride in refluxing CH\textsubscript{3}CN to give 9-tritylhyoxanthine (77%) after column chromatography. The 6-oxo function was replaced by imidazolyl via the modified Appel method. Detritylation in HOAc/H\textsubscript{2}O (9:1) at 60 °C followed by deprotonation and precipitation with CO\textsubscript{2} gave the product (65%).

![Scheme 3. Synthesis of 6-(imidazol-1-yl)purine (Method 2)](image-url)
2.1.3. 2-Chloro-6-(imidazol-1-yl)purine

Three different routes were developed for synthesis of 2-chloro-6-(imidazol-1-yl)purine. First, the 6-chloro function of 2,6-dichloropurine can be regioselectively replaced by imidazole in DMF at 65 °C to give the product in good yield (66%) as shown in Scheme 4. It is well known that the 6-chloride is much more readily replaced than the 2-Cl group. 39

Scheme 4. Synthesis of 2-chloro-6-(imidazol-1-yl)purine (Method 1)

The second route involved the preparation of 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-2,6-dichloropurine from guanosine, regioselective S_N,Ar substitution of the 6-Cl group with imidazole and acidic deglycosylation (Scheme 5). Acetylation of guanosine in DMF/pyridine at 75 °C gave 2’,3’,5’-tri-O-acetylguanosine (86%), which was transformed into 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-2-amino-6-chloropurine by treatment with POCl₃, BTEACl, and DMA in CH₃CN (87%). 40-41 Diazotization/chloro-dediazoniation gave 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-2,6-dichloropurine (99%). 42,43 Displacement of the 6-Cl group with excess imidazole in CH₃CN, followed by acidic deglycosylation gave the desired purine base (19) (72% for the three steps).
Scheme 5. Synthesis of 2-chloro-6-(imidazol-1-yl)purine (Method 2)

Significant solvent effects on the regioselectivity of the $S_{N}Ar$ replacement were observed, and replacement of the 2-Cl group also occurred in DMF (Scheme 6). The reaction in DMF at ambient temperature was incomplete after 15 h, and gave a product (62%) with traces of 9-(2,3,5-tri-O-acetyl-$\beta$-D-ribofuranosyl)-2,6-bis(imidazol-1-yl)purine and unreacted 17. The percentage of the disubstituted product increased with reaction time and elevated temperature (Table 1). In 1 h, only 9-(2,3,5-tri-O-acetyl-$\beta$-D-ribofuranosyl)-2-chloro-6-(imidazol-1-yl)purine was formed. The reaction in CH$_3$CN at ambient temperature was complete with excellent yield (87%). The solvent effects might be due to intermolecular hydrogen bonding between imidazole and DMF, which decreases the concentration of free imidazole (Figure 3). Addition of ZnCl$_2$ to activate the 6-Cl group did not affect the reaction significantly.
Scheme 6. Replacement of the 2-Cl group by imidazole in DMF

![Chemical Structures]

Figure 3. Intermolecular hydrogen bonding and its effect on the basicity of imidazole

Table 1. Solvent effects on the S_N_Ar displacement of chloride from 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-2,6-dichloropurine

<table>
<thead>
<tr>
<th>Imidazole (mol. eq.)</th>
<th>Temperature (°C)/time</th>
<th>Solvent</th>
<th>Monosubstitution %</th>
<th>Disubstitution %</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.0 + 3.0</td>
<td>65/(1.5 + 19 h)</td>
<td>DMF</td>
<td>25 (incomplete)</td>
<td>31</td>
</tr>
<tr>
<td>6.0</td>
<td>r.t./41 h</td>
<td>DMF</td>
<td>62 (incomplete)</td>
<td>Trace</td>
</tr>
<tr>
<td>6.0</td>
<td>r.t./19 h + 7 days (ZnCl_2)</td>
<td>DMF</td>
<td>64 (incomplete)*</td>
<td>None</td>
</tr>
<tr>
<td>12.0</td>
<td>r.t./31 h (DMF) + 5 days (DMF/CH_3CN)</td>
<td>DMF/CH_3CN</td>
<td>48 (complete)</td>
<td>None</td>
</tr>
<tr>
<td>18.0</td>
<td>r.t./50 h</td>
<td>CH_3CN</td>
<td>87 (complete)</td>
<td>None</td>
</tr>
</tbody>
</table>

* ZnCl_2 (3 molar equivalents) was added.

Table 1. Solvent effects on the S_N_Ar displacement of chloride from 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-2,6-dichloropurine
The third route also started with guanosine. Acetylation (86%) followed by tritylation of the 2-amino group gave 2’,3’,5’-tri-O-acetyl-2-N-tritylguanosine (98%). Replacement of the 6-oxo group with imidazole by the modified Appel method, followed by detritylation and diazotization/chloro-dediazoniation gave 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-2-chloro-6-(imidazol-1-yl)purine (33%). The relatively low yield resulted from multiple column separations to remove the byproduct Ph₃PO (Scheme 7). Acidic deglycosylation gave 2-chloro-6-(imidazol-1-yl)purine in modest overall yield.

Scheme 7. Synthesis of 2-chloro-6-(imidazol-1-yl)purine (Method 3)

2.1.4. 2-Amino-6-(imidazol-1-yl)purine and 2-Acetamido-6-(imidazol-1-yl)purine

A straightforward strategy for synthesis of 2-amino-6-(imidazol-1-yl)purine was S_NAr displacement of the 6-Cl group of 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-2-amino-6-chloropurine by imidazole, followed by deglycosylation (Scheme 8). A significant solvent effect was observed for the S_NAr reaction. Displacement in DMF at 65 °C caused partial deacetylation of the 3’-OAc group. Displacement in refluxing CH₃CN,
however, gave 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-2-amino-6-(imidazol-1-yl)purine as the only product in high yield (88%).

Scheme 8. Attempted synthesis of 2-amino-6-(imidazol-1-yl)purine

The solvent effect might also result from intermolecular hydrogen bonding between imidazole and DMF, and this association might increase the basicity of imidazole (Figure 3). Deglycosylation in AcCl/HOAc at 65 °C gave 2-acetylguanine, with loss of the 6-imidazole ring.

This strategy was then attempted for the preparation of 2-acetamido-6-(imidazol-1-yl)purine (Scheme 9). Guanosine was acetylated in pyridine to give 2-N,2′,3′,5′-O-tetraacetylguanosine (94%). A mixture of this compound, POCl₃, DMA and BTEACl was refluxed in CH₃CN to give 2-acetamido-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-6-chloropurine (90%). Displacement of the 6-Cl group with imidazole in CH₃CN at 70 °C gave 2-acetamido-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-6-(imidazol-1-yl)purine in excellent yield (95%). Deacetylation gave 2-amino-6-(imidazol-1-yl)-9-(β-D-ribofuranosyl)purine. However, deglycosylation of the peracetylated compound 28 in
AcCl/HOAc at 65 °C also gave 2-N-acetylguanine (25) with loss of the 6-imidazole ring and minor quantities of the desired product 29.

Scheme 9. Attempted synthesis of 2-acetamido-6-(imidazol-1-yl)purine

Removal of the 6-(imidazol-1-yl) ring in HOAc at 65 °C might be enhanced by intramolecular hydrogen bonding, which might increase the local concentration of acetate anion. Protonation of N1 and acetylation (R = Ac) or protonation (R = H) of imidazole might increase the attraction for negative charge and activate imidazole as a leaving group (Figure 4).

Figure 4. Possible mechanism for displacement of imidazole
The reactivity of the 6-Cl group for S_N_Ar displacement is influenced by the C2 substituents. It was observed that the reactivity decreased in the order: 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-2,6-dichloropurine >> 2-acetamido-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-6-chloropurine > 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-2-amino-6-chloropurine.

The failed deglycosylation step was subsequently circumvented. 9-Tritylguanine has been introduced for regiospecific N7 alkylation, and cleaved under mild conditions. Therefore, trityl protection of guanine was tried. Guanine was dissolved in 0.5 N NaOH/H_2O followed by precipitation with HCl/H_2O to pH ~10. This activated guanine was persilylated in HMDS with a catalytic amount of (NH_4)_2SO_4, and tritylated in CH_3CN at ambient temperature with 1.06 molar equivalents of trityl chloride. 9-Tritylguanine was the only product observed after desilylation in CH_2Cl_2/MeOH/H_2O overnight. However, with 2.12 molar equivalents of trityl chloride, some 2-N,9-bis(trityl)guanine was formed (4%).

It is advantageous for the preparation of 2-amino-6-(imidazol-1-yl)purine if both the 2-amino group and N9 are protected, as shown by the results for guanosine. Reaction conditions were tuned to prepare 2-N,9-bis(trityl)guanine (Table 2). Persilylated guanine was tritylated in refluxing CH_3CN with 1.06 molar equivalents of trityl chloride to give both 2-N-tritylguanine (28%) and 9-tritylguanine (41%) after desilylation. Therefore, 9-tritylguanine is the kinetic product, and 2-N-tritylguanine is the thermodynamic product due to its decreased steric repulsion. With a larger excess of trityl chloride, 2-N,9-bis(trityl)guanine was formed exclusively in refluxing CH_3CN (Scheme 10).
The 2-N,9-bis(trityl)guanine was transformed into 2-amino-6-(imidazol-1-yl)purine (69%) by replacement of the 6-oxo group with imidazole under the modified Appel conditions, followed by detritylation in TFA/H₂O (9:1) at ambient temperature, or HOAc/H₂O (9:1) at 60 °C (Scheme 11). Diacetylation in DMF at 150 °C (49%) followed by regioselective monodeacetylation (>93%) gave 2-acetamido-6-(imidazol-1-yl)purine.

<table>
<thead>
<tr>
<th>Entry</th>
<th>T °C</th>
<th>Ph₃CCl (mol. eq.)</th>
<th>9-tritylguanine %</th>
<th>2-N-tritylguanine %</th>
<th>2-N,9-bistryptyguanine %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>r.t.</td>
<td>1.06</td>
<td>73</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Reflux</td>
<td>1.19</td>
<td>41</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>r.t.</td>
<td>2.12</td>
<td>80</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>45</td>
<td>2.12</td>
<td>34</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>Reflux</td>
<td>2.12</td>
<td>?</td>
<td>?</td>
<td>31-33</td>
</tr>
<tr>
<td>6</td>
<td>Reflux</td>
<td>3.5</td>
<td>Trace</td>
<td>0</td>
<td>72</td>
</tr>
<tr>
<td>7</td>
<td>Reflux</td>
<td>4.12</td>
<td>Trace</td>
<td>0</td>
<td>72</td>
</tr>
<tr>
<td>8</td>
<td>Reflux</td>
<td>5.36</td>
<td>Trace</td>
<td>0</td>
<td>65</td>
</tr>
<tr>
<td>9</td>
<td>Reflux</td>
<td>6.0</td>
<td>0</td>
<td>0</td>
<td>62</td>
</tr>
</tbody>
</table>

Table 2. Tritylation of persilylated guanine
Scheme 10. Tritylation of guanine

Scheme 11. Synthesis of 2-amino-6-(imidazol-1-yl)purine and 2-acetamido-6-(imidazol-1-yl)purine
A more concise route to 2-acetamido-6-(imidazol-1-yl)purine might begin with 2-
N-acetylguanine. However, bis(tritylation) of 2-N-acetylguanine with excess trityl
chloride gave 2-N-acetyl-2-N,6-O-bis(trityl)guanine (Scheme 12). 9-Tritylguanine and 2-
N-acetyl-2-N,6-O-bis(trityl)guanine were obtained (after desilylation in
NH$_3$/H$_2$O/CH$_2$Cl$_2$) when 1.06 molar equivalents of trityl chloride were used.
Interestingly, 2-N-acetyl-2-N,6-O-bis(trityl)guanine was not deacetylated. Apparently the
2-N-acetyl group was effectively shielded by the two trityl groups.

![Scheme 12. Tritylation of 2-N-acetylguanine](image)

2.2. Regiospecific N9 Alkylation

It was reported that alkylation of 2-amino-6-(1,2,4-triazol-4-yl)purine gave 9-
alkyl-2-amino-6-(1,2,4-triazol-4-yl)purines regiospecifically.$^{24}$ We reasoned that
alkylation of 6-(1,2,4-triazol-4-yl)purine should also give 9-alkyl-6-(1,2,4-triazol-4-
yl)purines regiospecifically.
2.2.1. Alkylation of 6-(1,2,4-Triazol-4-yl)purine

The alkylation results with 6-(1,2,4-triazol-4-yl)purine (3) are given in Scheme 13. Treatment of the purine sodium salt in dried DMF with various alkyl iodides gave exclusively N9-alkylated products in excellent yields. Minor byproducts of 6-alkoxy-9-alkylpurines were formed due to the lability of the triazolyl function. Benzylation with BnCl gave only a moderate yield. Halogen exchange of BnCl to BnI, followed by treatment of the purine sodium salt with the BnI solution in CH₃CN gave 9-benzyl-6-(1,2,4-triazol-4-yl)purine exclusively in excellent yield.

![Chemical structure of 6-(1,2,4-triazol-4-yl)purine and its alkylated derivatives](image)

<table>
<thead>
<tr>
<th>R</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₃ (a)</td>
<td>90</td>
</tr>
<tr>
<td>CH₂CH₃ (b)</td>
<td>92</td>
</tr>
<tr>
<td>CH₃CH₂CH₂CH₂ (c)</td>
<td>84</td>
</tr>
<tr>
<td>Bn (d)</td>
<td>98 (BnI)</td>
</tr>
<tr>
<td></td>
<td>67 (BnCl)</td>
</tr>
<tr>
<td>Cyclopentyl (e)</td>
<td>87</td>
</tr>
<tr>
<td>2-Propyl (f)</td>
<td>99</td>
</tr>
<tr>
<td>1-Methylheptyl (g)</td>
<td>85</td>
</tr>
</tbody>
</table>

Scheme 13. Alkylation of 6-(1,2,4-triazol-4-yl)purine

The X-ray crystal data for 9-ethyl-6-(1,2,4-triazol-4-yl)purine indicated a nearly coplanar structure for the two linked heterocyclic rings with small dihedral angles as shown in Figure 5 [9-ethyl-6-(1,2,4-triazol-4-yl)purine: C5’- N4’ – C6-N1 ~4.0°, C3’-N4’ – C6-C5 ~5.8°]. An approximately coplanar structure was also observed for 9-
benzyl-6-(1,2,4-triazol-4-yl)purine (C5’-N4’– C6-N1 ~8.0°, C3’-N4’ – C6-C5 ~10.5°).

This larger deviation from a coplanar structure might be caused by the bigger alkyl function and correspondingly larger non-bonded intermolecular interactions in the crystal (Figure 6).

Figure 5. ORTEP diagram of 9-ethyl-6-(1,2,4-triazol-4-yl)purine

C5’-N4’ – C6-N1 ~4.0°, C3’-N4’ – C6-C5 ~5.8°

Figure 6. Crystal structure of 9-benzyl-6-(1,2,4-triazol-4-yl)purine

C5’-N4’ – C6-N1 ~8.0°, C3’-N4’– C6-C5 ~10.5°
2.2.2. Alkylation of 6-(imidazol-1-yl)purine

Alkylation of 6-(imidazol-1-yl)purine under the described conditions gave exclusively N9-alkylated products as shown by TLC and $^1$H NMR. After silica gel chromatographic purification, excellent yields were obtained for all the reactions (Scheme 14). Traces of 6-alkoxy-9-alkylpurine byproducts were also observed with extended reaction times due to the lability of the 6-imidazolyl function, although the 6-imidazolyl group is more stable than 6-triazolyl. Alkylation (Et, $i$-Pr, cyclopentyl) using K$_2$CO$_3$ as base in DMF gave exclusively N9-alkylated products with excellent yields, and no 6-alkoxy-9-alkylpurines were observed under these convenient conditions.

![Diagram](Image)

<table>
<thead>
<tr>
<th>R</th>
<th>Yields/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$ (a)</td>
<td>83</td>
</tr>
</tbody>
</table>
| CH$_2$CH$_3$ (b) | 95  
   87*  |
| CH$_3$CH$_2$CH$_2$CH$_2$ (c) | 92 |
| 2-Pr (d)   | 79        |
|           | 91*       |
| Cyclopentyl (e) | 92  
   98*  |
| Bn (f)     | 96        |

* K$_2$CO$_3$ was used instead of NaH

**Scheme 14. Alkylation of 6-(imidazol-1-yl)purine**

Benzylation of 6-(imidazol-1-yl)purine (9b) with excess BnI may occur at both N9 of the purine ring and N3’ of the imidazole ring (Scheme 15). Treatment of the
sodium salt of 6-(imidazol-1-yl)purine with 4.54 molar equivalents of BnI in CH$_3$CN/DMF gave the dibenzylated imidazolium salt exclusively. This salt was treated with 1 N methanolic sodium methoxide at ambient temperature to give 1-(9-benzylpurin-6-yl)-3-N-benzyl-2-methoxy-2,3-dihydroimidazole and 9-benzyl-6-methoxypurine.

Scheme 15. Benzylation at the imidazole ring
1-(Purin-6-yl)imidazolium chloride (9a) formed by acidic deglycosylation of 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-6-(imidazol-1-yl)purine (7) was alkylated directly to give poor yields of 9-alkyl-(6-imidazol-1-yl)purines (Scheme 16). The salt 9a was then deprotonated in 0.5 N aqueous NaOH. The needle-like crystalline sodium salt that precipitated was directly alkylated (EtI) to give 9-ethyl-6-(imidazol-1-yl)purine exclusively in high yields. NaH, added in DMF, lowered the yield of 9-ethyl-6-(imidazol-1-yl)purine and gave traces of 6-ethoxy-9-ethylpurine as a byproduct (Scheme 16).

The N9 regioisomer 39e was confirmed by NOESY (Figure 7). No cross peaks between the 6-imidazolyl hydrogens and those on the cyclopentyl group were observed, but H8/cyclopentyl showed a significant NOE.

Scheme 16. Effects of deprotonation and protonation of 6-(imidazol-1-yl)purine on alkylation
2.2.3. Alkylation of 2-Amino-6-(imidazol-1-yl)purine

Alkylations of 2-amino-6-(imidazol-1-yl)purine were not as clean as those with 6-(imidazol-1-yl)purine and 6-(1,2,4-triazol-4-yl)purine, and yields were lower (Scheme 17). Alkylation of the sodium salt of 2-amino-6-(imidazol-1-yl)purine with iodobutane occurred at both N9 and the 2-amino group to give 2-amino-9-butyl-6-(imidazol-1-yl)purine (63%) and 9-butyl-2-butylamino-6-(imidazol-1-yl)purine (7%). Alkylations [CH$_2$O(CH$_2$)$_2$OAc, Bu, cyclopentyl] using K$_2$CO$_3$ as the base in DMF gave exclusively N9-alkylated products in moderate to excellent yields (50, 81, 99%).
Scheme 17. Alkylation of 2-amino-6-(imidazol-1-yl)purine

Intermediates for the preparation of acyclovir were prepared by alkylation of both 2-amino-6-(imidazol-1-yl)purine and 2-acetamido-6-(imidazol-1-yl)purine in moderate yields (50 – 60%). Treatment of the sodium salt of 2-amino-6-(imidazol-1-yl)purine with BnI in CH$_3$CN gave 2-amino-9-benzyl-6-(imidazol-1-yl)purine as the only product in good yield (77%). X-Ray crystal data showed a coplanar structure for the two linked heterocyclic rings (C2’-N1’ – C6-N1 ~7.1°, C5’-N1’ – C6-C5 ~9.3°), and confirmed the regioselective N9 alkylation (Figure 8).
C2’-N1’ – C6-N1 ~7.1°, C5’-N1’ – C6-C5 ~9.3°

Figure 8. ORTEP diagram of 2-amino-9-benzyl-6-(imidazol-4-yl)purine

2.2.4. Alkylation of 2-Chloro-6-(imidazol-1-yl)purine

9-Alkyl-2-chloro-6-(imidazol-4-yl)purines were prepared by direct alkylation (Scheme 18). The sodium salt of 14 was alkylated with iodobutane, iodocyclopentane and benzyl iodide (0.3 N, CH₃CN) in DMF to give the 9-alkyl-2-chloro-6-(imidazol-4-yl)purines exclusively in high yields (84-87%).

| R                | Yield/|%
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₃CH₂CH₂CH₂     (a)</td>
<td>87</td>
</tr>
<tr>
<td>Bn* (b)</td>
<td>85</td>
</tr>
<tr>
<td>Cyclopentyl (c)</td>
<td>84</td>
</tr>
</tbody>
</table>

* 0.3 N BnI in CH₃CN was used.

Scheme 18. Alkylation of 2-chloro-(6-imidazol-1-yl)purine
2.3. Steric and Electronic Effects of the 6-Heteroaryl groups on Alkylations of Purines

The regiospecificity for N9 alkylation could result from both steric and electronic effects. The trend towards increased regioselectivity with increasing atom size with 6-halopurines is a good example of these effects (Table 3 and Figure 9).48

<table>
<thead>
<tr>
<th>6-Substituent</th>
<th>Atom radii/Å*</th>
<th>N9/N7</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>1.20</td>
<td>4</td>
</tr>
<tr>
<td>F</td>
<td>1.47</td>
<td>3.4</td>
</tr>
<tr>
<td>Cl</td>
<td>1.75</td>
<td>5.5</td>
</tr>
<tr>
<td>Br</td>
<td>1.85</td>
<td>7.3</td>
</tr>
<tr>
<td>I</td>
<td>1.98</td>
<td>9</td>
</tr>
</tbody>
</table>


Table 3. Effects of 6-halogen atom sizes on regioselectivity

Figure 9. Correlation between N7/N9 alkylation regioselectivity and 6-halogen atom sizes
The regioselectivity for alkylation of 6-alkylpurines also correlated well with average close contact distances (Figure 12, Table 4). The average close contact distance was defined in Eq. 1 as an effective indicator of steric volume. The minimized conformational energy \( E(\phi) \) and corresponding shortest distance \( r(\phi) \) between N7 and the closest hydrogen atom in the alkyl group at each torsional angle \( \phi \) were calculated using the MM2 method or at the RHF/6-31 G* level. The average close contact distance \( \langle r \rangle \) was then calculated according to Eq. 1. The results are given in Table 4 and Figures 10 and 11. The correlation between the calculated average close contact distances and regioselectivity is shown in Figure 12.

\[
\langle r \rangle = \frac{\int_0^{2\pi} r(\phi) \exp[-E(\phi)/RT]d\phi}{\int_0^{2\pi} \exp[-E(\phi)/RT]d\phi}
\]

(Eq. 1)

Thus, for the 6-(methyl, ethyl, and isopropyl)purines, steric hindrance dominates the alkylation regioselectivity. For the 6-halopurines (F, Cl, Br, I), steric hindrance dominates the selectivity with possible contributions from electronic effects. The high regioselectivity for N9 alkylation of the 6-heteroarylpurines may result significantly from steric hindrance.
Table 4. Average close contact distances \( \langle r \rangle \) based on theoretical calculations

<table>
<thead>
<tr>
<th>6-Substituent</th>
<th>( \langle r \rangle ) (H-N7)/( \text{Å}^0 )</th>
<th>N9/N7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Me*</td>
<td>2.902</td>
<td>9</td>
</tr>
<tr>
<td>Et*</td>
<td>2.762</td>
<td>16</td>
</tr>
<tr>
<td>( i-\text{Pr} )*</td>
<td>2.701</td>
<td>25</td>
</tr>
<tr>
<td>Imidazol-1-yl**</td>
<td>2.436</td>
<td>1/0</td>
</tr>
<tr>
<td>Triazol-4-yl**</td>
<td>2.470</td>
<td>1/0</td>
</tr>
</tbody>
</table>

* MM2; ** RHF/6-31 G*

Figure 10. Minimized conformational energies based on MM2 calculations
Figure 11. Examples of calculated minimum conformational energies and close contact distances
Figure 12. Correlation between the calculated average close contact distances and regioselectivity for N7/N9 alkylation

As X-ray crystal data indicated, 6-(pyrrol-1-yl)purines were coplanar or almost coplanar.\textsuperscript{4,5,7} A small deviation from coplanarity was observed for \textit{cis}-methyl 1-N-[\textit{t}-butoxycarbonyl]amino]-2-[6-(pyrrol-1-yl)purin-9-yl]cyclopropanecarboxylate (dihedral angle, 9.4°) due to the large N9 substituent.\textsuperscript{8} 4-(1,2,4-Triazol-1-yl)pyrimidine was also shown to be coplanar. The crystal structure of 2‘,3‘,5‘-tri-O-acetyl-4-(1,2,4-triazol-1-yl)uridine showed a very small dihedral angle between the triazolyl and uracil rings (3.1°).\textsuperscript{49} Theoretical calculations indicate that 6-(pyrrol-1-yl), 6-(2-furyl), 6-(1,2,4-triazol-4-yl) and 6-(imidazol-1-yl)purines are all coplanar, with 6-(2-thienyl) as an exception (Figure 13). MM2 calculations gave a dihedral angle of 20.4° for 6-(2-thienyl)purine. The crystal structure of 6-phenyl-4-(2-thienyl)-\textit{IH}-pyrimidin-2-one also showed deviation from a coplanar structure (dihedral angle, 15.9°).\textsuperscript{50} The nonplanar conformation might be caused by the longer C-S bonds and the larger atomic size of sulfur.
6-(2-Thienyl)purine 6-(2-Furyl)purine

C5-C6 – C2’-S1’, 20.4° (MM2) coplanar (MM2)

6-(Pyrrol-1-yl)purine              6-(Imidazol-1-yl)purine       6-(1,2,4-triazol-4-yl)purine

\[ d_{N7-H}: \begin{align*}
    & 2.3652 \text{ Å (RHF/6-31G*)} \\
    & 2.4223 \text{ Å (RHF/6-31G*)} \\
    & 2.4605 \text{ Å (RHF/6-31G*)}
\end{align*} \]

*Figure 13. The most stable conformations of 6-heteroarylpurines*
Figure 14. Steric energy barriers of 6-heteroarylpurines calculated at the RHF/6-31 G* level

The results of RHF/6-31 G* calculations of rotational barriers between the two heterocyclic rings of 6-(1,2,4-triazol-4-yl)purine, 6-(imidazol-1-yl)purine and 6-(pyrrol-1-yl)purine are shown in Figure 14. The rotation angle between the two rings is predicted to be $0^\circ$ for the global minimum energy conformers. The conformer with maximum energy has the two rings perpendicular. The conformational thermodynamics are governed by the competition between unfavorable non-bonded interactions, such as exchange repulsions, and attractive $\pi$-conjugation effects. The hydrogen-hydrogen and lone pair-lone pair repulsive interactions are of the same order of magnitude, and much larger than hydrogen-lone pair repulsions. High rotational barriers were predicted (9.5–11.2 kcal·mol$^{-1}$), which result from the strong $\pi$-conjugation and weak repulsive interactions. This is in harmony with the shorter bond length of C6-N1’ with a smaller
dihedral angle between the two rings (Figure 15). Lopez and coworkers reported a systematic study of the conformations of 2-phenylaziridines.\textsuperscript{51,52} It was shown that the preferred conformation for 2-phenylaziridine had the phenyl ring nearly bisecting the plane of the aziridine ring, with maximum conjugation. As steric hindrance due to substituents on the rings increased, the phenyl ring rotated towards a perpendicular plane.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{diagram.png}
\caption{Dependence of the bond length (C6-N1') on conformations of 6-heteroarylpurines}
\end{figure}

* RHF/6-31 G*/\BY3LP/6-31+ G*

** RHF/6-31 G*

Computations performed on two conjugated ring systems such as 6-6' (two 6-membered rings), 6-5 (one 6-membered ring and one 5-membered ring), and 5-5' (two 5-
membered rings) predicted that the conformers with global minimum energies for 6-6’ and 6-5 are twisted, but coplanar for 5-5’ systems.\textsuperscript{53,54} Cui et al. have studied N-aryl cyclic imides using \textit{ab initio} calculations.\textsuperscript{53} They found the most stable conformer for \textit{N}-phenylmaleimide was twisted with a torsional angle of 46°, whereas the \textit{N}-(pyrrol-2-yl)maleimide and \textit{N}-(pyrrol-3-yl)maleimide ring systems were coplanar with low rotational barriers of about 2 kcal·mol\textsuperscript{-1}. Russo et al. have investigated the conformational behavior of isomeric phenylpyrroles at the STO-3G level.\textsuperscript{54} It was shown that the energy maxima corresponded to perpendicular conformers and that the energy minima had nonplanar conformers with a flat torsional potential between 0 – 45°. The energy barrier height at 90° was larger for 2-phenylpyrrole than for 3-phenylpyrrole (15.7 kJ·mol\textsuperscript{-1} versus 12.4 kJ·mol\textsuperscript{-1}) with better conjugation corresponding to the \(\pi\)-bond order (0.274 versus 0.228).

With the 6-heteroarylpurines [6-(imidazol-1-yl), 6-(1,2,4-triazol-4-yl) and 6-(pyrrol-1-yl)], steric hindrance due to the hydrogen-lone pair interaction at N7 is small. N7 is bent away from C6 in the fused purine ring system. The conformer with global minimum energy is coplanar, governed by strong conjugation, which agrees well with the X-ray crystal data. Small deviations from coplanar conformations observed in crystal structures result from intermolecular crystal-packing interactions.\textsuperscript{55}

In the coplanar conformations of the 6-heteroarylpurines, the N7 atom is shielded by the H2’/H5’ atoms of the 6-(imidazol-1-yl) or the H3’/H5’ atoms of the 6-(1,2,4-triazol-4-yl) rings. The high regioselectivity for N9 alkylation of 6-heteroarylpurines likely results from this effective guidance. Experimental evidence supported the hypothesis that decreasing the shielding of N7 of the purine ring by H2’ of an attached
imidazole with a large substituent at C5’ decreased the regioselectivity (Figure 16, Scheme 19). Treatment of the sodium salt of 2-chloro-6-(4,5-diphenylimidazol-1-yl)purine (48) with EtI gave both N9 and N7 alkylated products (N9/N7, 5:1). Structures of the N9/N7 isomers (50/51) were confirmed by NOE effects (Figures 17,18).

Alkylation of the sodium salt of 2-chloro-6-(2-butylimidazol-1-yl)purine (49) with EtI gave exclusively the N9 alkylated product 52 (Scheme 18). The conformer with global minimum energy for 2-chloro-6-(2-butylimidazol-1-yl)purine should be coplanar as shown by the crystal structure of 6-(2-hexylimidazol-1-yl)purine (Figure 19).

![Chemical structures](image)

**Fig 16. Twisted conformation of 6-(4,5-diphenylimidazol-1-yl)purine (MM2)**

<table>
<thead>
<tr>
<th>Ar</th>
<th>N9/N7</th>
<th>Yield%</th>
</tr>
</thead>
<tbody>
<tr>
<td>4,5-diphenylimidazol-1-yl (48)</td>
<td>5:1</td>
<td>100</td>
</tr>
<tr>
<td>2-butylimidazol-1-yl (49)</td>
<td>1:0</td>
<td>100</td>
</tr>
</tbody>
</table>

**Scheme 19. Twisted conformation lowers the selectivity for N9 alkylations**
Figure 17. NOE for 2-chloro-7/9-ethyl-6-(4,5-diphenylimidazol-1-yl)purine

Figure 18. NOE difference spectra for 2-chloro-7-ethyl-6-(4,5-diphenylimidazol-1-yl)purine
C2’-N1’–C6-N1 ~3.1°, C5’-N1’–C6-C5 ~6.4°

Figure 19. ORTEP diagram of 6-(2-hexylimidazol-1-yl)purine

6-(Pyrrrol-1-yl)purine has the shortest H2’–N7 distance among the 6-[(imidazol-1-yl), (1,2,4-triazol-4-yl) and (pyrrol-1-yl)]purines. However, its reported regioselectivity for N9 alkylation is the lowest. The difference may have resulted from the harsh conditions in refluxing DMF. In conclusion, both the coplanar conformation and conjugative electronic effects might contribute to the regiospecificity of N9 alkylation for 6-(imidazol-1-yl) and 6-(1,2,4-triazol-4-yl)purines.

2.4. Biological assays

The parent and alkylated 6-heteroarylpurines were evaluated by Professor E. De Clercq (Rega Institute for Medical Research, Katholieke Universiteit Leuven) against herpes simplex virus-1 (KOS), herpes simplex virus-1 (TK’KOS ACV’), herpes simplex virus-2 (G), vaccinia virus and vesicular stomatitis virus in HEL cell cultures, vesicular stomatitis virus, coxsackie virus (B4) and respiratory syncytial virus in HeLa cell cultures, parainfluenza-3 virus, reovirus, sindbis virus, Coxsackie virus (B4) and Punta Toro virus in Vero cell cultures, and HIV-1 and HIV-2 in human T-lymphocyte (CEM) cells. The preliminary studies showed the enantiomeric mixture of 9-(1-methylheptyl)-6-
(1,2,4-triazol-4-yl)purine inhibits the growth of vaccinia virus (MIC = 24 µg/mL) with relatively low cytotoxicity (MCC = 200 µg/mL). It also showed weak antiviral activity against HIV-1 and HIV-2. 2-Chloro-9-cyclopentyl-6-(imidazol-1-yl)purine and 2-chloro-6-(2-propylimidazol-1-yl)purine had antiviral activities against vesicular stomatitis virus, coxsackie virus (B4), and respiratory syncytial virus but with similar level of cytotoxicity. 2-Chloro-6-(2-propylimidazol-1-yl)purine acts as a inhibitor against both HIV-1 and HIV-2. 9-Butyl-2-butylamino-6-(imidazol-1-yl)purine showed inhibition against parainfluenza-3 virus, reovirus, sindbis virus, Coxsackie virus (B4) and Punta Toro virus in Vero cell culture, but with almost the same cytotoxicity.

The compounds were also evaluated for their cytostatic activity against murine leukemia cells (L1210/0), human T-lymphocyte cells (Molt4/C8, CEM/0), and against MSV-induced transformation of C3H/3T3 embryo murine fibroblasts in vitro. Antitumor activities against the proliferation of murine leukemia cells (L1210/0) was observed for the enantiomeric mixture of 9-(1-methylheptyl)-6-(1, 2, 4-triazol-4-yl)purine (IC$_{50}$ = 86 ± 30 µM) and 2-chloro-6-(2-propylimidazol-1-yl)purine (IC$_{50}$ = 22 ± 7 µM). Antitumor activities against the human T-lymphocyte cells (Molt4/C8 and CEM/0) were observed for 9-(1-methylheptyl)-6-(1, 2, 4-triazol-4-yl)purine (IC$_{50}$ = 48 ± 10 µM and IC$_{50}$ = 56 ± 2 µM, respectively), 2-chloro-9-cyclopentyl-6-(imidazol-1-yl)purine (IC$_{50}$ = 40 ± 7 µM and IC$_{50}$ = 53 ± 6 µM, respectively) and 2-chloro-6-(2-propylimidazol-1-yl)purine (IC$_{50}$ = 9.1 ± 2.2 µM and IC$_{50}$ = 9.6 ± 1.5 µM, respectively). The enantiomeric mixture of 9-(1-methylheptyl)-6-(1,2,4-triazol-4-yl)purine, 2-amino-9-benzyl-6-(imidazol-1-yl)purine, 2-chloro-9-cyclopentyl-6-(imidazol-1-yl)purine, 2-chloro-9-butyl-6-(imidazol-1-yl)purine, 9-benzyl-2-chloro-6-(imidazol-1-yl)purine and 2-chloro-6-(i-propylimidazol-1-yl)purine
also showed inhibitory effects against MSV-induced transformation of C3H/3T3 embry
murine fibroblasts.

3. Conclusions

Alkylation of 6-heteroarylpurines (6-triazolyl and 6-imidazolyl) gave 9-alkyl-6-
heteroarylpurines exclusively. Both steric and electronic effects contribute to the
regioselectivity. The steric effects result from the coplanar conformations of the two
joined heterocyclic rings, which are governed by conjugation. Several compounds
showed antiviral and antitumor activities.

4. Experimental Section

6-(1,2,4-Triazol-4-yl)purine (3)

A suspension of \( N,N' \)-dimethylformamide azine dihydrochloride (9.09 g, 42.3
mmol) and adenine (4.05 g, 30 mmol) in dried DMF (200 mL) was refluxed for 22 h. The
mixture was cooled and volatiles were evaporated. MeOH and toluene were added and
evaporated (3 x). The residue was suspended in MeOH. Filtration and drying gave 3
(5.32 g, 95%): \(^1\)H NMR (500 MHz, DMSO-\( d_6 \)) \( \delta \) 14.08 (s, 1H), 9.66 (s, 2H), 8.89 (s, 1H),
8.80 (s, 1H); \(^{13}\)C NMR (125 MHz, DMSO-\( d_6 \)) \( \delta \) 154.8, 151.7, 146.1, 142.1, 140.8, 121.5.

2-Amino-6-(1,2,4-triazol-4-yl)purine (4)

A suspension of \( N,N' \)-dimethylformamide azine dihydrochloride (95 mg, 0.425
mmol) and 2,6-diaminopurine (50 mg, 0.325 mmol) in dried DMF (12.5 mL) was
refluxed for 10 h. TLC of the reaction mixture showed two major spots, both of which
gave identical \(^1\)H NMR data. The more rapidly migrating spot was contaminated with
unidentified byproducts, which were monocyclized (N2) and/or bis-cyclized (N2 and N6)
compounds depending on the reaction conditions. The mixture was cooled and volatiles
were evaporated. Methanol and toluene were added and evaporated (3 x). The residue was suspended in methanol. Filtration and drying gave 4 (49 mg, 72%). \(^1\)H NMR (500MHz, DMSO-\(d_6\)) \(\delta\) 13.00 (s, 1H), 9.42 (s, 2H), 8.23 (s, 1H), 6.78 (s, 2H); HRMS m/z 202.0706 (M\(^+\) [C\(_7\)H\(_6\)N\(_8\)] = 202.0715), in agreement with published data.\(^{24}\) The presumed byproduct 6-amino-2-(1,2,4-triazol-4-yl)purine had: \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) \(\delta\) 13.00 (s, 1H), 9.06 (s, 2H), 8.13 (s, 1H), 7.72 (s, 2H).

2',3',5'-Tri-\(O\)-acetylinosine (6)

To an ice-cold mixture of inosine (7.2 g, 26.9 mmol) in pyridine (50 mL) was added Ac\(_2\)O (20 mL, 21.5 g, 211 mmol). The mixture was allowed to warm to ambient temperature, and stirred for 15 h. Volatiles were evaporated in vacuo, and the residue was dissolved in CH\(_2\)Cl\(_2\)/H\(_2\)O. The organic phase was washed (H\(_2\)O, 30 mL; NaHCO\(_3\)/H\(_2\)O, 3 x 30 mL; brine, 2 x 30 mL) and dried (Na\(_2\)SO\(_4\)). Volatiles were evaporated to give a solid (10.1 g, 95%). Recrystallization (MeOH) gave 6: \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 8.32 (s, 1H), 8.04 (s, 1H), 6.19 (d, \(J = 5.1\) Hz, 1H), 5.90 (‘t’, \(J = 5.4\) Hz, 1H), 5.63 (dd, \(J = 4.7, 5.3\) Hz, 1H), 4.36–4.49 (m, 3H), 2.172 (s, 3H), 2.165 (s, 3H), 2.12 (s, 3H), in agreement with published data.\(^{56}\)

9-(2, 3, 5-Tri-\(O\)-acetyl-\(\beta\)-D-ribofuranosyl)-6-(imidazol-1-yl)purine (7)

A suspension of 6 (1.9 g, 5 mmol), Ph\(_3\)P (3.2 g, 12 mmol), I\(_2\) (2.7 g, 10.4 mmol), and imidazole (1.3 g, 18 mmol) in toluene (50 mL) and DIPEA (4.4 mL, 3.27 g, 25.2 mmol) was stirred at 95 °C overnight. Volatiles were evaporated, and the residue was extracted with boiling EtOAc. The combined EtOAc extracts were evaporated to dryness. The residue was chromatographed [EtOAc/hexanes/HOAc (70:30:1) → EtOAc → EtOAc/HOAc (49:1)] to give 7 (1.64 g, 77%): \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 9.20 (‘t’, \(J\)
= 0.9 Hz, 1H), 8.84 (s, 1H), 8.42 (‘t’, J = 1.5 Hz, 1H), 8.30 (s, 1H), 7.29 (‘d’, J = 0.6 Hz, 1H), 6.30 (d, J = 5.3 Hz, 1H), 6.01 (‘t’, J = 5.3 Hz, 1H), 5.71 (‘t’, J = 5.2 Hz, 1H), 4.43–4.53 (m, 3H), 2.21 (s, 3H), 2.18 (s, 3H), 2.13 (s, 3H); HRMS m/z 445.1475 (MH\(^+\) \[C_{19}H_{22}N_6O_7\] = 445.1472), in agreement with published data.\(^{26}\)

**6-(Imidazol-1-yl)purine (9b)**

Method 1. A suspension of 6 (4.0 g, 10 mmol), Ph₃P (6.4 g, 24 mmol), I₂ (5.3 g, 20.7 mmol), and imidazole (2.5 g, 36 mmol) in toluene (100 mL) and DIPEA (8.8 mL, 6.53 g, 50.5 mmol) was stirred at 95 °C overnight. Volatiles were evaporated in vacuo, and the residue was extracted with boiling EtOAc. The combined EtOAc extracts were evaporated to dryness. The residue was dissolved in HOAc (400 mL), and AcCl [4.2 mL, 4.64 g, 58 mmol (5.8 eq., 0.14 M)] was added. The mixture was stirred at 65 °C overnight (reaction complete, TLC). The solution was concentrated to 100 mL, and cooled. The precipitated solid was collected by filtration, and the filter cake was washed with HOAc and CH₂Cl₂ to give 6-(imidazol-1-yl)purine·HCl (1.78 g, 80%).

(1) Recrystallization from methanol gave the protonated compound: mp 296-297 °C (dec.); \(^1\)H NMR (300 MHz, DMSO-\(d_6\)) \(\delta\) 10.01 (d, J = 1.8 Hz, 1H), 8.96 (s, 1H), 8.88 (s, 1H), 8.73 (s, 1H), 7.85 (s, 1H); \(^{13}\)C NMR (75 MHz, DMSO-\(d_6\)) \(\delta\) 156.0, 152.3, 147.5, 143.3, 137.1, 124.1, 122.7, 119.8; HRMS m/z 186.0673 (M\(^+\) [C₈H₆N₆] = 186.0654).

(2) The precipitated solid from the HOAc solution was dissolved in 0.5 M NaOH/H₂O, and needle-like crystals precipitated from the solution after standing at ambient temperature. The solid was collected by filtration, and recrystallized from MeOH/Et₂O to give the sodium salt as a white solid: \(^1\)H NMR (200 MHz, DMSO-\(d_6\)) \(\delta\) 9.15 (t, J = 1.1 Hz, 1H), 8.44 (t, J = 1.3 Hz, 1H), 8.40 (s, 1H), 8.04 (s, 1H), 7.13–7.14 (m, 1H).
(3) The precipitated solid from the HOAc solution was dissolved in 0.5 M NaOH/H₂O, and needle-like crystals precipitated from solution after standing at ambient temperature. The solid was collected by filtration, and washed with CO₂/H₂O to give 9b: UV (MeOH) max 211, 281, 290 nm (ε 22 000, 15 100, 11 900), min 234, 288 nm (ε 3300, 11 800); ¹H NMR (200 MHz, DMSO-<i>d</i>₆) δ 13.90 (br s, 1H), 9.09–9.10 (m, 1H), 8.82 (s, 1H), 8.72 (s, 1H), 8.42–8.44 (m, 1H), 7.26–7.27 (m, 1H).

Method 2. A suspension of 9-tritylhypoxanthine (3.0 g, 8 mmol), Ph₃P (11.9 g, 45 mmol), I₂ (11.3 g, 44 mmol), and imidazole (2.9 g, 42 mmol) in toluene (240 mL) and DIPEA (7.1 mL, 5.27 g, 41 mmol) was stirred at 95 °C overnight. Volatiles were evaporated, and the residue was extracted with boiling EtOAc (2 x 100 mL). The combined EtOAc extracts were evaporated to dryness. Both the original residue and the solid obtained by extraction with EtOAc were treated with HOAc/H₂O (9:1, 150 mL) and stirred at 60 °C for 20 h. Volatiles were evaporated in vacuo from the two solutions, and the residues were dissolved in NaOH/H₂O (0.1 N), and washed (CH₂Cl₂). Precipitation with CO₂, and filtration gave 9b (0.35 g and 0.55 g, repectively). The combined mother liquors were evaporated to dryness, and the residue was extracted (NaOH/H₂O) and precipitated with CO₂ to give a second crop (0.03 g, 65% total).

9-Tritylhypoxanthine (11)

A suspension of hypoxanthine (2.0 g, 14.7 mmol) and (NH₄)₂SO₄ (0.46 g) was stirred in HMDS (300 mL) under reflux for 15 h. Volatiles were evaporated from the resulting clear solution in vacuo, and the residue was dissolved in dried CH₃CN (150 mL). Trityl chloride (8.81 g, 30 mmol) was added, and the clear solution was stirred under reflux for 48 h. Volatiles were evaporated in vacuo, and the residue was dissolved
in CH₂Cl₂ (50 mL). NH₃/H₂O (28-30% w/w, 80 mL) was added, and precipitation was observed immediately. The turbid mixture was stirred at ambient temperature overnight. Volatiles were evaporated in vacuo, and the residue was washed (H₂O, CH₂Cl₂) to give a solid (3.02 g). The combined organic layers were evaporated to dryness, and the residue was chromatographed (MeOH/CH₂Cl₂, 1:60 → 1:30) to give another crop of the compound (0.59 g, 65% total) after washing (CH₂Cl₂): ¹H NMR (300 MHz, DMSO-d₆) δ 12.23 (s, 1H), 7.72 (s, 1H), 7.65 (s, 1H), 7.30–7.37 (m, 9H), 7.13 (d, J = 7.0 Hz, 6H); ¹³C NMR (75 MHz, DMSO-d₆) δ 157.5, 150.2, 145.0, 141.9, 140.7, 130.0, 128.8, 128.4, 126.6, 75.8; HRMS m/z 401.1390 (MNa⁺ [C₂₄H₁₈N₄ONa] = 401.1378). Mono- and bistritylated byproducts were observed: LRMS (FAB) m/z 643 (MNa⁺ [C₄₃H₃₂N₄ONa] = 643).

Reaction of a mixture of two regiosomers of monotritylated products under the Appel conditions, followed by detritylation gave 9b as the sole product.

**2-Chloro-6-(imidazo-1-yl)purine (14)**

Method 1. 2,6-Dichloropurine (0.38 g, 2 mmol) and imidazole (0.82 g, 12.1 mmol) were dissolved in freshly distilled DMF (36 mL), and the mixture was stirred at 65 °C for ~20 h. Volatiles were evaporated, and the residue was washed with a large amount of CH₂Cl₂ to give 14 (0.29 g, 66%).

Method 2. Compound 18 (1.32 g, 2.75 mmol) was dissolved in HOAc (114 mL), and AcCl (1.14 mL, 1.26 g, 16.0 mmol) was added to the solution. The mixture in a sealed flask was stirred at 65 °C for 11 h, and volatiles were evaporated in vacuo. The residue was washed (CH₂Cl₂) and dissolved in 0.1 N NaOH/H₂O. Precipitation with CO₂ gave a solid (0.51 g, 83 %). Recrystallization (MeOH) gave 14 (0.35 g, 58%): UV (MeOH) max
218, 288, 298 nm (ε 27 300, 14 100, 13 300), min 241, 262, 295 nm (ε 5 800, 6 400, 12 000); ¹H NMR (500 MHz, DMSO-d₆) δ 14.09 (br s, 1H), 9.03 (s, 1H), 8.72 (s, 1H), 8.34 (s, 1H), 7.26 (s, 1H); ¹³C NMR (75 MHz, DMSO-d₆) δ 157.0, 152.4, 146.8, 145.7, 137.7, 131.4, 121.4, 118.1; HRMS m/z 220.0275 (M⁺ [C₈H₅ClN₆] = 220.0264).

9-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-2-amino-6-chloropurine (16)

The procedure to prepare 16 was analogous to the reported procedure.⁵⁷ POCl₃ (11.6 mL, 19.08 g, 124.5 mmol) was added to a stirred, refluxing solution (110 °C oil bath) of 2’,3’,5’-tri-O-acetylguanosine (8.47 g, 20.7 mmol), BTEACl (9.43 g, 41.4 mmol) and DMA (2.7 mL, 2.58 g, 21.3 mmol) in CH₃CN (140 mL), and the mixture was stirred at reflux for 25 min. Volatiles were evaporated in vacuo, and the residue was dissolved in CHCl₃ (200 mL). Ice was added, and the mixture was stirred for 15 min. The organic phase was washed [ice-H₂O (2 x 100 mL) and NaHCO₃/H₂O (2 x 100 mL)], and dried (Na₂SO₄). The solution was evaporated to dryness, and the residue was recrystallized (i-PrOH) to give 16 (7.74 g, 87%): ¹H NMR (500 MHz, CDCl₃) δ 7.88 (s, 1H), 6.01 (d, J = 5.0 Hz, 1H), 5.96 (t, J = 5.0 Hz, 1H), 5.75 (t, J = 5.0 Hz, 1H), 5.22 (br s, 2H), 4.36–4.47 (m, 3H), 2.15 (s, 3H), 2.11 (s, 3H), 2.10 (s, 3H); ¹³C NMR (75 MHz, DMSO-d₆) δ 170.8, 170.1, 170.0, 160.6, 154.4, 150.6, 142.0, 124.2, 85.5, 80.4, 72.6, 70.9, 63.6, 21.2, 21.1, 20.9; LRMS (FAB) m/z 450 (MNa⁺ [C₁₆H₁₈ClN₅O₇Na] = 450).

9-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-2,6-dichloropurine (17)

Compound 17 was prepared by a reported method.⁴² Chromatography (EtOAc/hexanes, 3:7 → 1:1) gave a white solid (1.48 g, 83%). Recrystallization from MeOH gave 17 (1.34 g, 77%): mp 161.5-162.5 °C (Lit.⁴²,⁴³ mp 158 °C); UV (MeOH) max 213, 253, 273 nm (ε 20 200, 4300, 8200), min 231, 257 nm (ε 1600, 4200); ¹H
NMR (500 MHz, CDCl₃) δ 8.31 (s, 1H), 6.23 (d, J = 5.5 Hz, 1H), 5.80 (t, J = 5.5 Hz, 1H), 5.58 (dd, J = 5.5, 4.2 Hz, 1H), 4.42–4.51 (m, 3H), 2.18 (s, 3H), 2.15 (s, 3H), 2.10 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 170.5, 169.8, 169.6, 153.7, 152.8, 152.6, 144.0, 131.6, 86.7, 81.1, 73.5, 70.8, 63.1, 21.1, 20.8, 20.6; HRMS (EI) m/z 446.0400 (M⁺ [C₁₆H₁₆Cl₂N₄O₇] = 446.0396).

The reaction was repeated. The derived residue was dissolved in CH₂Cl₂ (200 mL), and the resulting solution was washed (NaHCO₃/H₂O, 3 x 50 mL) and dried (Na₂SO₄). Volatiles were evaporated in vacuo, and the residue was recrystallized (i-PrOH, 2 x) to give yellow crystals of 17 (5.47 g, 76%). The residue derived from the mother liquor was chromatographed (EtOAc/hexanes, 1:1) to give an additional amount of 17 (1.62 g, 99% total).

9-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-2-chloro-6-(imidazol-1-yl)purine (18)

Method 1. Compound 17 (1.71 g, 3.83 mmol) and imidazole (4.71 g, 68.9 mmol) were dissolved in CH₃CN (75 mL) and stirred at ambient temperature under N₂ for 1.5 days (reaction complete, TLC). After removal of volatiles, the residue was chromatographed (MeOH/CH₂Cl₂,1:95 ~1:90) to give 18 (1.54 g, 94%): ¹H NMR (500 MHz, CDCl₃) δ 9.14 (s, 1H), 8.34 (s, 1H), 8.28 (s, 1H), 7.26 (s, 1H), 6.27 (d, J = 5.8 Hz, 1H), 5.83 (t, J = 5.7 Hz, 1H), 5.61 (dd, J = 4.3, 5.5 Hz, 1H), 4.44–4.52 (m, 3H), 2.19 (s, 3H), 2.18 (s, 3H), 2.11 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 170.5, 169.9, 169.7, 154.8, 154.4, 146.9, 143.0, 138.0, 131.4, 122.1, 117.6, 86.5, 81.0, 73.5, 70.9, 63.2, 21.1, 20.8, 20.6; HRMS (EI) m/z 501.0908 (M⁺ [C₁₉H₁₉ClN₆O₇Na] = 501.0901), 478.0987 (M⁺ [C₁₉H₁₉ClN₆O₇] = 478.1003).
Method 2. TMSCl (1.5 mL x 2, 2.57 g, 23.6 mmol) was added dropwise to a stirred solution of crude 22 (0.57 g, 1.24 mmol) and BTEANO$_2$ (0.89 g, 3.7 mmol) in dry CH$_2$Cl$_2$ (40 mL) under N$_2$. The solution was stirred at ambient temperature overnight, and the reaction mixture was diluted with CHCl$_3$ (200 mL). The solution was washed (NaHCO$_3$/H$_2$O) and dried (Na$_2$SO$_4$). Volatiles were evaporated in vacuo, and the residue was chromatographed (EtOAc/hexanes, 7:3) to give 18 (0.48 g, 81%).

9-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-2,6-bis(imidazol-1-yl)purine (19)

Compound 17 (0.23 g, 0.5 mmol) and imidazole (205 mg, 3.0 mmol) were dissolved in DMF (10 mL) and stirred at 65 °C under N$_2$ for 15 h (reaction incomplete, TLC). Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH$_2$Cl$_2$, 1:90 → 1:45) to give a mixture of 18 (0.06 g, 25%) and 19 (0.08 g, 31%). 19: $^1$H NMR (300 MHz, CDCl$_3$) δ 9.21 (s, 1H), 8.77 (s, 1H), 8.39 (1s, 1H), 8.28 (s, 1H), 8.06 (s, 1H), 7.723 (s, 1H), 13 (s, 1H), 6.22 (d, $J = 3.9$ Hz, 1H), 6.08 (t, $J = 4.8$ Hz, 1H), 5.79 (t, $J = 6.0$ Hz, 1H), 5.46–5.54 (m, 2H), 4.35 (dd, $J = 4.5$, 12.6 Hz, 1H), 2.19 (s, 3H), 2.16 (s, 3H), 2.02 (s, 3H); HRMS m/z 510.1609 (M$^+$ [C$_{22}$H$_{22}$N$_8$O$_7$] = 510.1611).

2',3',5'-Tri-O-acetylguanosine (20)

Compound 20 was prepared by a reported method.$^{57}$ Yield (9.98 g, 86%): $^1$H NMR (500 MHz, DMSO-$d_6$) δ 10.74 (s, 1H), 7.93 (s, 1H), 6.54 (br s, 2H), 5.98 (d, $J = 6.5$ Hz, 1H), 5.79 (t, $J = 6.0$ Hz, 1H), 5.49 (dd, $J = 4.0$, 6.0 Hz, 1H), 4.24–4.39 (m, 3H), 2.11 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 170.8, 170.2, 170.0, 157.4, 151.9, 136.4, 117.6, 85.1, 80.3, 72.8, 71.1, 63.8, 21.3, 21.1, 20.9; LRMS (FAB) m/z 432 (MNa$^+$ [C$_{16}$H$_{19}$N$_5$O$_8$Na] = 432), 454 (MNa$_2$ – H [C$_{16}$H$_{18}$N$_5$O$_8$Na$_2$] = 454).
2',3',5'-Tri-O-acetyl-2-N-tritylguanosine (21)

A mixture of 20 (1.0 g, 2.45 mmol), Ph3CCl (2.32 g, 8.3 mmol), EtN(i-Pr)2 (1.45 mL, 1.076 g, 8.3 mmol) in pyridine (40 mL) was stirred under a N2 atmosphere for 20 h (reaction almost complete, TLC). Volatiles were evaporated in vacuo, and toluene was added and evaporated. The residue was dissolved in CH2Cl2, and the solution was washed (H2O, brine) and dried (Na2SO4). Volatiles were evaporated, and the residue was chromatographed (MeOH/CH2Cl2, 1:30) to give 21 as a white solid (1.55 g, 98%): 1H NMR (500 MHz, DMSO-d6) δ 10.72 (s, 1H), 7.86 (s, 1H), 7.70 (s, 1H), 7.21–7.30 (m, 15H), 5.28–5.32 (m, 2 H), 5.03 (t, J = 5.0 Hz, 1H), 4.00–4.14 (m, 3H), 2.08 (s, 3H), 2.04 (s, 3H), 1.95 (s, 3H); 13C NMR (125 MHz, DMSO-d6) δ 170.2, 169.4, 169.0, 156.5, 151.5, 149.9, 144.7, 136.0, 128.7, 127.9, 126.9, 117.4, 84.1, 79.0, 71.8, 70.5, 69.9, 63.2, 20.7, 20.5, 20.3; HRMS m/z 696.2062 (MNa2 – H [C35H32N5O8Na2] = 696.2046).

9-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-2-amino-6-(imidazol-1-yl)purine (22)

Method 1. A mixture of 21 (1.46 g, 2.24 mmol), I2 (2.84 g, 11.2 mmol), Ph3P (2.94 g, 11.2 mmol) and imidazole (0.76 g, 11.2 mmol) was stirred in toluene (60 mL) at 95 °C for 15 min. DIPEA (3.9 mL, 2.89 g, 22.4 mmol) was added, and the mixture was stirred at 95 °C overnight. After removal of volatiles, the residue was extracted with boiling EtOAc (100 mL + 10 mL x 3). The combined EtOAc extracts were evaporated to dryness, and the residue was dried under vacuum. This material was stirred in TFA/H2O (9:1, 60 mL) at 0 °C for 1.5 h. Volatiles were evaporated in vacuo, and the residue was chromatographed (CH2Cl2 → MeOH/CH2Cl2, 1:30) to give 22 as a colored solid. This material was dissolved in MeOH, charcoal was added, and the mixture was heated and filtered. The filtrate was evaporated to dryness, and the residue was dissolved in CH2Cl2.
The resulting solution was washed (NaHCO$_3$/H$_2$O, brine) and dried (Na$_2$SO$_4$). Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH$_2$Cl$_2$, 1:20) to give a colored solid (0.57 g, 56%): $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 9.06 (s, 1H), 8.29 (s, 1H), 7.88 (s, 1H), 7.22 (s, 1H), 6.06 (d, $J = 4.9$ Hz, 1H), 6.00–6.02 (m, 1H), 5.80–5.82 (m, 1H), 5.16 (s, 2H), 4.41–4.50 (m, 3H), 2.17 (s, 3H), 2.13 (s, 6H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 170.8, 169.9, 169.8, 159.6, 155.2, 146.5, 140.3, 137.6, 130.5, 117.6, 117.2, 86.7, 80.06, 73.09, 70.7, 63.2, 20.9, 20.8, 20.7; HRMS m/z 482.1387 (MNa$^+$ [C$_{19}$H$_{21}$N$_7$O$_7$Na] = 482.1400).

Method 2. A mixture of 16 (213 mg, 0.5 mmol) and imidazole (615 mg, 9 mmol) in CH$_3$CN (10 mL) was stirred at reflux under N$_2$ for 12 h (reaction complete, TLC). Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH$_2$Cl$_2$, 1:60) to give 22 as a solid (193 mg, 88%).

This reaction was incomplete in DMF, and gave partially deacetylated starting material. With added ZnCl$_2$ (2.4 mol. eq.), the reaction in DMF was less than half complete.

2-N',2',3','5'-O-Tetraacetylguanosine (26)

A mixture of guanosine (2 g, 7.1 mmol) and Ac$_2$O (15 mL, 16.2 g, 159 mmol) in pyridine (30 mL) was stirred at 65 °C for 24 h. Volatiles were evaporated in vacuo, and the residue was chromatographed twice (MeOH/CH$_2$Cl$_2$, 1:30; EtOAc) to give 26 (2.98 g, 94%): $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 11.96 (s, 1H), 9.13 (s, 1H), 7.72 (s, 1H), 5.95 (d, $J = 5.2$ Hz, 1H), 5.92 (t, $J = 5.2$ Hz, 1H), 5.73–5.75 (m, 1H), 4.66–4.0 (m, 1H), 4.48–4.51 (m, 2H), 2.34 (s, 3H), 2.17 (s, 3H), 2.11 (s, 6H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 172.1,
171.9, 170.0, 169.6, 155.6, 147.6, 147.5, 138.7, 123.0, 87.7, 80.3, 73.1, 71.3, 63.5, 24.5, 21.2, 20.8, 20.7; HRMS (FAB) m/z 451.1336 (M^+ [C_{18}H_{21}N_5O_9] = 451.1339).

2-Acetamido-9-(2, 3, 5-tri-O-acetyl-β-D-ribofuranosyl)-6-chloropurine (27)

The procedure was developed from a reported method. Both DMA and CH₃CN were dried over CaH₂ and distilled freshly before use. POCl₃ (8 mL, 13.2 g, 85.8 mmol) was added to a stirred and refluxing solution (110 °C oil bath) of 26 (5.38 g, 11.9 mmol), BTEACl (5.5 g, 24.1 mmol) and DMA (1.6 mL, 1.53 g, 12.6 mmol) in CH₃CN (130 mL). The mixture was stirred at reflux for 6 h. Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH₂Cl₂, 1:97) to give 27 (5.03 g, 90%). ^1H NMR (500 MHz, CDCl₃) δ 8.39 (s, 1H), 8.13 (s, 1H), 6.10 (d, J = 4.3 Hz, 1H), 5.91 (t, J = 5.0 Hz, 1H), 5.80 (br s, 1H), 4.41–4.53 (m, 3H), 2.45 (s, 3H), 2.16 (s, 3H), 2.11 (s, 6H); ^13C NMR (125 MHz, CDCl₃) δ 170.6, 169.9, 169.8, 152.3, 152.2, 151.9, 143.2, 128.9, 87.6, 80.6, 73.6, 70.7, 63.4, 25.4, 21.0, 20.8, 20.7; HRMS m/z 492.0910 (MNa^+ [C_{18}H_{20}N_5O_8Na] = 492.0898).

2-Acetamido-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-6-(imidazol-1-yl)-purine (28)

A mixture of 27 (118 mg, 0.25 mmol) and imidazole (315 mg, 4.63 mmol) in CH₃CN (5 mL) was stirred at ambient temperature under N₂ for 12 h. The temperature was elevated to 75 °C, and the reaction was almost complete (TLC) after another 17 h. Volatiles were evaporated in vacuo, and the residue was dissolved in CH₂Cl₂ and washed (H₂O). The organic phase was dried (Na₂SO₄), and volatiles were evaporated. The residue was chromatographed (MeOH/CH₂Cl₂, 1:95 → 1:30) to give 28 (115 mg, 94%): ^1H NMR (500 MHz, CDCl₃) δ 9.08 (s, 1H), 8.39 (s, 1H), 8.30 (s, 1H), 8.11 (s, 1H), 7.25 (s, 1H), 6.13 (d, J = 4.6 Hz, 1H), 5.94 (t, J = 4.9 Hz, 1H), 5.78 (br s, 1H), 4.43–4.54 (m,
3H), 2.55 (s, 3H), 2.17 (s, 3H), 2.13 (s, 6H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 170.7, 169.8, 169.7, 154.3, 152.7, 146.4, 142.4, 137.8, 131.2, 119.8, 117.7, 87.4, 80.5, 73.6, 70.7, 63.4, 25.5, 21.1, 20.8, 20.7; HRMS m/z 501.1598 (M$^+$ [C$_{21}$H$_{23}$N$_7$O$_8$] = 501.1608).

A similar reaction in DMF was incomplete.

**2-Acetamido-6-(imidazol-1-yl)purine (29)**

Compound 35 (0.56 g, 2.0 mmol) was refluxed in MeOH (30 mL) for 7 days (traces of 35, TLC). Volatiles were evaporated to give 29 (0.463 g, 97%): $^1$H NMR (500 MHz, DMSO-$d_6$) δ 13.70 (br s, 1H), 10.62 (s, 1H), 9.00 (s, 1H), 8.53 (s, 1H), 8.32 (s, 1H), 7.25 (s, 1H), 2.24 (s, 3H); HRMS m/z 243.0869 (M$^+$ [C$_{10}$H$_9$N$_7$O] = 243.0869).

Compound 35 (0.37 g, 1.3 mmol) was refluxed in EtOH (50 mL) for 24 h to give 29 (0.30 g, 93%) with traces of 35. The 9-acetyl group can also be selectively removed with NaHCO$_3$/H$_2$O at ambient temperature, but not in NH$_3$/H$_2$O (28-30% w/w).

Other attempts. To a solution of 28 (251 mg, 0.5 mmol) in HOAc (21 mL) was added AcCl (0.21 mL, 0.232 g, 2.95 mmol), and the mixture was stirred at 65 ºC for 17 h (precipitation observed, reaction complete, TLC). Volatiles were evaporated in vacuo, and the residue was washed (CH$_2$Cl$_2$) to give a solid mixture (112 mg). This material was dissolved in 0.1 N NaOH/H$_2$O, and precipitated with CO$_2$ to give a mixture of 2-N-acetylguanine and 29 (11:2.6).

The reaction was repeated. The precipitated solid was filtered, and washed (CH$_2$Cl$_2$) to give crude 2-N-acetylguanine.

Compound 23 was treated with AcCl/HOAc as described. Precipitation occurred, and the solid was filtered and washed (CH$_2$Cl$_2$) to give crude 2-N-acetylguanine.
9-Tritylguanine (31)

Guanine was dissolved in 0.5 N NaOH/H₂O, and then precipitated by addition of HCl/H₂O to pH ~10 (lower pH resulted in much longer times for silylation). This activated guanine was dried, ground to a fine powder, and dried further before use.

Freshly activated guanine (153 mg, 1 mmol) and (NH₄)₂SO₄ (18 mg) were stirred in HMDS (15 mL) under reflux for 28 h to give a clear solution. Volatiles were evaporated in vacuo, and the residue was dissolved in dried CH₃CN (10 mL). Trityl chloride (285 mg, 1.02 mmol) in CH₃CN (4 mL) was added, and the clear solution was stirred at ambient temperature for 48 h. Volatiles were evaporated in vacuo, and CH₂Cl₂/MeOH/H₂O (1:1:1, 15 mL) was added to give a turbid solution, which was stirred at ambient temperature overnight. Volatiles were evaporated in vacuo, and the residue was washed with H₂O, then CH₂Cl₂ to give 31 (291 mg, 73%): ¹H NMR (500 MHz, DMSO-d₆) δ 10.49 (s, 1H), 7.29–7.37 (m, 9H), 7.18 (s, 1H), 7.11 (d, J = 8 Hz, 6H), 6.02 (br s, 2H); HRMS m/z 394.1669 (MH⁺ [C₂₄H₂₀N₅O] = 394.1668).

2-N,9-Bistritylguanine (32)

Freshly activated guanine (0.45 g, 3 mmol) and (NH₄)₂SO₄ (60 mg) were stirred in HMDS (50 mL) under reflux for 24 h to give a clear solution. Volatiles were evaporated in vacuo, and the residue was dissolved in dried CH₃CN (50 mL). Trityl chloride (3.5 g, 12.6 mmol) was added, and the solution was stirred under reflux for 48 h. Volatiles were evaporated in vacuo, and the residue was dissolved in CH₂Cl₂ (10 mL). NH₃/H₂O (28-30 %, 30 mL) was added, and precipitation was observed immediately. The mixture was stirred at ambient temperature overnight. Volatiles were evaporated in vacuo, and the residue was washed (H₂O, CH₂Cl₂) to give 32 as a solid (1.37 g, 72%).
which was further purified by dissolving in MeOH/CH$_2$Cl$_2$ (1:15) and filtering: $^1$H NMR (500 MHz, DMSO-$_d$$_6$) $\delta$ 10.75 (s, 1H), 7.35 (s, 1H), 7.08–7.19 (m, 19H), 6.87 (d, $J = 7.4$ Hz, 6H), 6.81 (d, $J = 7.3$ Hz, 6H); $^{13}$C NMR (125 MHz, DMSO-$_d$$_6$) $\delta$ 157.3, 151.8, 151.0, 145.3, 142.4, 139.6, 129.6, 128.8, 128.5, 128.3, 127.6, 126.9, 120.3, 75.4, 71.1; HRMS $m/z$ 635.2675 (M$^+ [C_{43}H_{33}N_5O] = 635.2685$).

2-N-Tritylguanine (33)

Freshly activated guanine (450 mg, 3 mmol) and (NH$_4$)$_2$SO$_4$ (53 mg) were stirred in HMDS (50 mL) under reflux for 36 h. Volatiles were evaporated in vacuo, and the residue was dissolved in dried CH$_3$CN (50 mL). Trityl chloride (998 mg, 3.58 mmol) was added, and the clear solution was stirred at reflux for 20 h. Volatiles were evaporated in vacuo, and NH$_3$/H$_2$O//MeOH (30 mL/5 mL) was added to give a turbid solution, which was stirred at ambient temperature overnight. Volatiles were evaporated in vacuo, and the residue was washed (H$_2$O, CH$_2$Cl$_2$) to give solid 31 (475 mg, 41%). The organic phase was combined and evaporated to dryness, and the residue was chromatographed (MeOH/CH$_2$Cl$_2$, 1:20) to give 33 (327 mg, 28%): $^1$H NMR (500 MHz, DMSO-$_d$$_6$) $\delta$ 12.28 (s, 1H), 10.53 (s, 1H), 7.58 (s, 1H), 7.46 (s, 1H), 7.46–7.20 (m, 15H); LRMS (FAB) $m/z$ 416 (MNa$^+$ [C$_{24}$H$_{19}$N$_5$ONa] = 416), 438 (MNa$_2$ – H [C$_{24}$H$_{18}$N$_5$ONa$_2$] = 438).

2-Amino-6-(imidazol-1-yl)purine (34)

A mixture of 32 (1.90 g, 3 mmol), I$_2$ (3.88 g, 15 mmol), Ph$_3$P (3.99 g, 15 mmol) and imidazole (1.10 g, 15 mmol) was stirred in toluene (150 mL) at 95 °C for 15 min, and DIPEA (2.9 mL, 2.15 g, 16.6 mmol) was added. The mixture was stirred at 95 °C overnight. After removal of volatiles, the residue was boiled with EtOAc (3 x) and filtered hot. The combined EtOAc extracts were evaporated to dryness. The residue was
dissolved in TFA/H$_2$O (9:1, 60 mL), and the solution was stirred at 0 °C for 4 h. Volatiles were evaporated in vacuo, and the residue was dissolved in 0.1 N NaOH/H$_2$O/CH$_2$Cl$_2$ (100 mL/100 mL). The organic layer was extracted with 0.1 N NaOH/H$_2$O (50 mL x 2), and the aqueous phase was combined, washed [CH$_2$Cl$_2$ (2 x 50 mL)], and neutralized with CO$_2$. Volatiles were evaporated in vacuo, and the residue was washed (H$_2$O, CH$_2$Cl$_2$) to give 34 (0.40 g, 69%): UV (MeOH) max 222, 320 nm (ε 29 800, 8700), min 207, 280 nm (ε 16 100, 1500); $^1$H NMR (500 MHz, DMSO-$d_6$) δ 12.89 (s, 1H), 8.94 (s, 1H), 8.25 (s, 1H), 8.16 (s, 1H), 7.18 (s, 1H), 6.67 (s, 2H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 160.7, 157.5, 145.4, 141.9, 137.2, 130.5, 117.7, 115.4; HRMS m/z 201.0753 (M$^+$ [C$_8$H$_7$N$_7$] = 201.0763).

2-Acetamido-9-acetyl-6-(imidazol-1-yl)purine (35)

To a suspension of 34 (1.0 g, 5.0 mmol) in DMF (15 mL) was added Ac$_2$O (2.4 mL, 2.59 g, 25.4 mmol). The mixture was stirred at 150 °C for 4 h, and precipitation occurred. The mixture was cooled in a refrigerator overnight. The solid was filtered and washed (CH$_2$Cl$_2$) to give 35 (698 mg, 49%): $^1$H NMR (500 MHz, DMSO-$d_6$) δ 10.88 (s, 1H), 8.98 (s, 1H), 8.94 (s, 1H), 8.29 (s, 1H), 7.27 (s, 1H), 2.96 (s, 3H), 2.28 (s, 3H); HRMS m/z 285.0979 (M$^+$ [C$_{12}$H$_{11}$N$_7$O$_2$] = 285.0974).

2-$N$-Acetyl-2-$N$,6-O-ditritylguanine (37)

A mixture of 2-acetamidoguanine (588 mg, 3 mmol) and (NH$_4$)$_2$SO$_4$ (48 mg) was stirred in HMDS (50 mL) under reflux for 24 h to give a clear solution. Volatiles were evaporated in vacuo, and the residue was dissolved in dried CH$_3$CN (50 mL). Trityl chloride (1.73 g, 6.2 mmol) was added, and the clear solution was stirred at ambient temperature for 15 h. Precipitation occurred, and the solid was filtered, washed
(CH$_3$CN), and stirred in NaHCO$_3$/H$_2$O/MeOH overnight. Volatiles were evaporated in vacuo, and the residue was washed (H$_2$O, CH$_2$Cl$_2$) to give 37 (1.12 g, 54%): $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 12.46 (s, 1H), 7.82 (s, 1H), 7.05–7.29 (m, 30H), 1.44 (s, 3H); HRMS $m/z$ 700.2675 (MNa$^+$ [C$_{45}$H$_{35}$N$_5$O$_2$Na] = 700.2688), 722.2519 (MN$_2$Na – H C$_{45}$H$_{34}$N$_5$O$_2$Na$_2$] = 722.2508).

9-Alkyl-6-(1, 2, 4-triazol-4-yl)purine (38a-f)

General method 1. A solution of 3 (0.19 g, 1.0 mmol) in DMF (5 mL) was treated with sodium hydride (0.06 g, 60% w/w suspension, 1.44 mmol), and the reaction mixture was stirred at ambient temperature under N$_2$ for 1 h. The respective iodoalkane was added to the solution, and the resulting mixture was stirred until alkylation was completed as indicated by TLC. Traces of products resulting from nucleophilic replacement of triazole by alkoxides derived from the iodoalkanes were observed.

9-Methyl-6-(1,2,4-triazol-4-yl)purine (38a)

The sodium salt of 3 (0.19 g, 1.0 mmol) was treated with iodomethane (0.23 mL, 0.21 g, 1.5 mmol) by general method 1. Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH$_2$Cl$_2$, 1:14) to give a solid. This material was washed (H$_2$O) to give 38a (0.18 g, 90%): UV (MeOH) max 210, 277 nm ($\varepsilon$ 27 300, 12 700), min 235 nm ($\varepsilon$ 2600); $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 9.67 (s, 2H), 8.95 (s, 1H), 8.79 (s, 1H), 3.93 (s, 3H), LRMS (EI) $m/z$ 201 (M$^+$ [C$_8$H$_7$N$_7$] = 201).

For structure confirmation, compound 38a was stirred in Me$_2$NH/H$_2$O (40% w/w, 20 mL) at ambient temperature until the displacement was complete (TLC). Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH$_2$Cl$_2$, 1:40) to give 9-methyl-6-$N,N$-dimethylaminopurine (0.15 g, 86%): $^1$H NMR (300 MHz,
CDCl$_3$) $\delta$ 8.30 (s, 1H), 7.64 (s, 1H), 3.74 (s, 3H), 3.47 (br s, 6H); LRMS (EI) $m$/z 177 ($M^+$ [C$_8$H$_{11}$N$_5$] = 177).

**9-Ethyl-6-(1,2,4-triazol-4-yl)purine (38b)**

The sodium salt of 3 (0.19 g, 1.0 mmol) was treated with iodoethane (0.12 mL, 0.23 g, 1.5 mmol) by general method 1. Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH$_2$Cl$_2$, 1:40 $\rightarrow$ 1:30 $\rightarrow$ 1:20) to give a solid (0.228 g). This material was dissolved in CH$_2$Cl$_2$ and washed (H$_2$O). The aqueous phase was extracted with CH$_2$Cl$_2$. The organic layer was combined and dried (Na$_2$SO$_4$). Volatiles were evaporated in vacuo to give a solid (0.205 g, 92%). Recrystallization (CH$_2$Cl$_2$/hexanes) gave 38b: mp 218.5-220.5 °C; UV (MeOH) max 212, 277 nm (ε 25 400, 13 100), min 235 nm (ε 2600); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 9.67 (s, 2H), 8.88 (s, 1H), 8.25 (s, 1H), 4.46 (q, $J$ = 7.3 Hz, 1H), 1.66 (t, $J$ = 7.3 Hz, 1H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 154.2, 152.4, 145.3, 143.4, 141.2, 122.8, 39.8, 15.6; HRMS (EI) $m$/z 215.0911 ($M^+$ [C$_9$H$_9$N$_7$] = 215.0919). Anal. Calcd for C$_9$H$_9$N$_7$: C, 50.23; H, 4.22; N, 45.56. Found: C, 50.45; H, 4.32; N, 45.70.

**9-Butyl-6-(1,2,4-triazol-4-yl)purine (38c)**

The sodium salt of 3 (0.19 g, 1.0 mmol) was treated with iodobutane (0.17 mL, 27 mg, 1.5 mmol) by general method 1. Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH$_2$Cl$_2$, 1/40) to give a solid (207 mg, 84%). Recrystallization (CH$_2$Cl$_2$/hexanes) gave 38c: mp 154-155 °C; UV(MeOH) max 211, 277 nm (ε 26 300, 13 100), min 235 nm (ε 2200); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 9.67 (s, 2H), 8.88 (s, 1H), 8.20 (s, 1H), 7.29 (s, 1H), 4.39 (t, $J$ = 7.2 Hz, 2H), 1.98 (quint, $J$ = 7.4 Hz, 2H), 1.43 (sext, $J$ = 7.3 Hz, 2H), 1.02 (t, $J$ = 7.3 Hz, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$
9-Benzyl-6-(1,2,4-triazol-4-yl)purine (38d)

1. Benzyl chloride as the alkylating reagent. The sodium salt of 3 (0.19 g, 1.0 mmol) was treated with benzyl chloride (0.17 mL, 0.19 g, 1.5 mmol) by general method 1. Volatiles were evaporated in vacuo, and the residue was dissolved in CH$_2$Cl$_2$/H$_2$O. The aqueous layer was extracted with CH$_2$Cl$_2$. The organic phase was combined and dried (Na$_2$SO$_4$). Volatiles were evaporated, and the residue was dissolved in a limited amount of CH$_2$Cl$_2$, and precipitated with Et$_2$O. The solid was filtered to give 38d (0.18 g, 67%).

2. Benzyl iodide as the alkylating reagent. To a clear solution of NaI (0.45 g, 3 mmol) in CH$_3$CN (3 mL) was added BnCl (0.17 mL, 1.9 g, 1.5 mmol). The solution was stirred at ambient temperature, and the precipitation of NaCl occurred. The resulting solution of BnI was used without further purification. The sodium salt of 3 (0.19 g, 1.0 mmol) was alkylated with this benzyl iodide solution by general method 1. Volatiles were evaporated, and the residue was chromatographed (MeOH/CH$_2$Cl$_2$, 1:20) to give a solid (0.276 g, 98%). Recrystallization (CH$_2$Cl$_2$/hexanes) gave compound 38d: mp 218-218.8 °C; UV (MeOH) max 211, 277 nm (ε 33 300, 13 700), min 238 nm (ε 3200); $^1$H NMR (300 MHz, CDCl$_3$) δ 9.66 (s, 2H), 8.91 (s, 1H), 8.20 (s, 1H), 7.42–7.29 (m, 5H), 5.55 (s, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 154.4, 152.8, 145.6, 143.5, 141.1, 134.7, 129.6, 129.5, 129.3, 128.3, 122.6, 48.1; HRMS (EI) m/z 277.1075 (M$^+$ [C$_{14}$H$_{11}$N$_7$] = 277.1076). Anal. Calcd for C$_{14}$H$_{11}$N$_7$: C, 60.64; H, 4.00; N, 35.36. Found: C, 60.81; H, 3.98; N, 35.30.
9-Cyclopentyl-6-(1,2,4-triazol-4-yl)purine (38e)

The sodium salt of 3 (0.19 g, 1.0 mmol) was treated with iodocyclopentane (0.3 mL, 509 mg, 2.6 mmol) by general method 1 (reaction almost complete, TLC). Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH₂Cl₂, 1:40) to give a solid (0.226 g, 87%). Recrystallization (CH₂Cl₂/hexanes) gave 38e: mp 181-182.5 °C; UV (MeOH) max 211, 278 nm (ε 28 900, 13 700), min 236 nm (ε 3000); ¹H NMR (300 MHz, CDCl₃) δ 9.66 (s, 2H), 8.86 (s, 1H), 8.26 (s, 1H), 5.10 (quint, J = 7.3 Hz, 1H), 2.46–2.34 (m, 2H), 2.17–1.84 (m, 6H); ¹³C NMR (75 MHz, CDCl₃) δ 154.4, 152.1, 144.2, 143.3, 141.2, 123.1, 57.1, 32.9, 24.2; HRMS (EI) m/z 255.1228 (M⁺ [C₁₂H₁₃N₇] = 255.1232). Anal. Calcd for C₁₂H₁₃N₇: C, 56.46; H, 5.13; N, 38.41. Found: C, 56.70; H, 5.18; N, 38.40.

9-Isopropyl-6-(1,2,4-triazol-4-yl)purine (38f)

The sodium salt of 3 (0.197 g, 1.0 mmol) was treated with 2-iodopropane (0.5 mL, 0.85 g, 5.0 mmol) by general method 1. Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH₂Cl₂, 1:25) to give a solid (0.239 g, 99%). Recrystallization (CH₂Cl₂/hexanes) gave 38f: mp 221-223.5 °C; UV (MeOH) max 211, 277 nm (ε 29 600, 12 800), min 239 nm (ε 2800); ¹H NMR (300 MHz, CDCl₃) δ 9.66 (s, 2H), 8.86 (s, 1H), 8.28 (s, 1H), 5.03 (sept, J = 6.8 Hz, 1H), 1.73 (d, J = 6.8 Hz, 6H); ¹³C NMR (75 MHz, CDCl₃) δ 154.0, 152.1, 144.2, 143.3, 141.2, 123.1, 48.4, 22.8; HRMS (EI) m/z 229.1069 (M⁺ [C₁₀H₁₁N₇] = 229.1076). Anal. Calcd for C₁₀H₁₁N₇: C, 56.46; H, 5.13; N, 42.77. Found: C, 56.70; H, 4.89; N, 42.70.

6-Isoproxy-9-isopropylpurine was formed as a byproduct from replacement of triazol-4-yl by isoproxyde formed in situ: ¹H NMR (300 MHz, CDCl₃) δ 8.52 (s, 1H),
7.97 (s, 1H), 5.67 (sept, J = 9.3 Hz, 1H), 4.89 (sept, J = 10 Hz, 1H), 1.64 (d, J = 10 Hz, 6H), 1.49 (d, J = 9.3 Hz, 6H); LRMS (EI) m/z 220 (M^+ [C_{11}H_{16}N_4O] = 220).

**9-(1-Methylheptyl)-6-(1,2,4-triazol-4-yl)purine (38g)**

The sodium salt of 3 (0.19 g, 1.0 mmol) was treated with 2-iodooctane (0.5 mL, 0.67 g, 3.1 mmol) by general method 1 (reaction almost complete, TLC). Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH_2Cl_2, 1:50) to give a solid racemic mixture (0.253 g, 85%): UV (MeOH) max 210, 277 nm (ε 3 1400, 15 300), min 238 nm (ε 4600); ^1H NMR (300 MHz, CDCl_3) δ 9.66 (s, 2H), 8.84 (s x 2, 1H), 8.24 (s x 2, 1H), 4.84 (sext, J = 6.8 Hz, 1H), 1.96–2.09 (m, 2H), 1.68 (d x 2, J = 1.8, 6.8 Hz, 3H), 1.23–1.30 (m, 8H), 0.83 (t, J = 6.8 Hz, 3H); ^1H NMR (500 MHz, DMSO-d_6) δ 9.66 (s, 2H), 8.95 (s, 1H), 8.92 (s x 2, 1H), 4.80–4.84 (m, 1H), 2.06–2.10 (m, 1H), 1.89–2.05 (m, 1H), 1.60 (“d”, J = 7.0 Hz, 3H), 1.18–1.26 (m, 7H), 1.04–1.05 (m, 1H), 0.79–0.82 (m, 3H); ^13C NMR (500 MHz, DMSO-d_6) δ 153.5, 151.5, 146.3, 142.5, 140.9, 122.3, 51.9, 35.3, 31.0, 28.1, 25.5, 21.9, 20.4, 13.8; LRMS (EI) m/z 299 (M^+ [C_{13}H_{21}N_7] = 299); (FAB) m/z 300 (MH^+ [C_{13}H_{22}N_7] = 300), 322 (MNa^+ [C_{13}H_{21}N_7Na] = 322). Byproduct isomers resulting from impurities in the iodoctane were observed.

**9-Alkyl-6-(imidazol-1-yl)purine (39a-f)**

General method 2. A solution of 9b (93 mg, 0.5 mmol) in DMF (5 mL) was treated with sodium hydride (23 mg, 60% w/w suspension, 0.6 mmol), and the suspension was stirred at ambient temperature under N_2 for 1 h. The respective iodoalkane was added, and the resulting mixture was stirred until the alkylation was completed as indicated by TLC.
General method 3. To a suspension of $9b$ (93 mg, 0.5 mmol) and $K_2CO_3$ (0.23 g, 1.5 mmol) in DMF (5 mL) was added the respective iodoalkane. The mixture was stirred overnight.

6-(Imidazol-1-yl)-9-methylpurine (39a)

The sodium salt of $9b$ (95 mg, 0.5 mmol) was treated with iodomethane (0.12 mL, 112 mg, 7.9 mmol) by general method 2. Volatiles were evaporated in vacuo, and the residue was dissolved in $H_2O/CH_2Cl_2$ (20 mL/50 mL). The aqueous layer was extracted ($CH_2Cl_2$, 2 x 20 mL). The organic layer was combined, washed ($H_2O$, 2 x 20 mL) and dried ($Na_2SO_4$). Volatiles were evaporated in vacuo to give $39a$ (86 mg, 83%). $^1H$ NMR (300 MHz, CDCl$_3$) $\delta$ 9.21 (d, $J = 1.8$ Hz, 1H), 8.84 (s, 1H), 8.43 (t, $J = 2.1$ Hz, 1H), 8.13 (s, 1H), 7.28 (d, $J = 0.9$ Hz, 1H), 3.99 (s, 3H); LRMS $m/z$ 200 ($M^+ [C_9H_8N_6] = 200$).

9-Ethyl-6-(imidazol-1-yl)purine (39b)

The sodium salt of $9b$ (93 mg, 0.5 mmol) was treated with iodoethane (0.06 mL, 117 mg, 0.75 mmol) by general method 2. Volatiles were evaporated in vacuo, and the residue was chromatographed ($MeOH/CH_2Cl_2$, 1:40) to give a solid (0.101 g, 95%). Recrystallization ($CH_2Cl_2/hexanes$) gave $39b$ (97 mg, 91%): mp 125.5-127 °C; UV (MeOH) max 212, 282, 292 nm ($\epsilon$ 24 000, 15 900, 11 800), min 235, 290 nm ($\epsilon$ 2900, 11 700); $^1H$ NMR (300 MHz, CDCl$_3$) $\delta$ 9.18 (s, 1H), 8.79 (s, 1H), 8.40 (t, $J = 1.2$ Hz, 1H), 8.13 (s, 1H), 7.25 (d, $J = 0.5$ Hz, 1H), 4.39 (q, $J = 7.3$ Hz, 2H), 1.61(t, $J = 7.3$ Hz, 3H); $^{13}C$ NMR (75 MHz, CDCl$_3$) $\delta$153.8, 152.3, 145.86, 144.17, 137.87, 130.90, 122.76, 117.58, 39.56, 15.64; HRMS $m/z$ 214.0959 ($M^+ [C_{10}H_{10}N_6] = 214.0967$). Anal. Calcd for $C_{10}H_{10}N_6$: C, 56.07; H, 4.70; N, 39.23. Found: C, 56.31; H, 4.52; N, 39.08.
Extended reaction times and excess iodoethane resulted in the formation of 6-ethoxy-9-ethytpurine: \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 8.55 (s, 1H), 7.94 (s, 1H), 4.68 (q, \(J = 7.1\) Hz, 2H), 4.32 (q, \(J = 7.3\) Hz, 1H), 1.57 (t, \(J = 7.3\) Hz, 3H), 1.54 (t, \(J = 7.3\) Hz, 3H); LRMS \(m/z\) 192 (M\(^+\) [C\(_9\)H\(_{12}\)N\(_4\)O] =192), 177 (M – CH\(_3\) [C\(_8\)H\(_9\)N\(_4\)O] = 177).

The reaction was repeated by general method 3. Compound 9b (98 mg, 0.5 mmol) was treated with iodoethane (80 µL, 159 mg, 1.0 mmol). Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH\(_2\)Cl\(_2\), 1:40) to give 39d (98 mg, 87%).

9-Butyl-6-(imidazol-1-yl)purine (39c)

The sodium salt of 9b (96 mg, 0.5 mmol) was treated with iodobutane (85.3 µL, 137 mg, 0.75 mmol) by general method 2. Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH\(_2\)Cl\(_2\), 1:40) to give a solid (114 mg, 92%). Recrystallization (CH\(_2\)Cl\(_2\)/hexanes) gave 39c: mp 86.5-87.5 °C; UV (MeOH) max 212, 282, 292 nm (\(\varepsilon\) 24 600, 17 100, 13 500), min 236, 290 nm (\(\varepsilon\) 3900, 12 900); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 9.21 (s, 1H), 8.82 (s, 1H), 8.43 (s, 1H), 8.13 (s, 1H), 7.29 (s, 1H), 4.36 (t, \(J = 7.3\) Hz, 2H), 1.97 (quint, \(J = 7.3\) Hz, 2H), 1.43 (sext, \(J = 7.3\) Hz, 2H), 1.02 (t, \(J = 7.3\) Hz, 3H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 154.0, 152.4, 145.9, 144.6, 137.9, 131.0, 122.7, 117.6, 44.3, 32.2, 20.1, 13.7; HRMS \(m/z\) 242.1265 (M\(^+\) [C\(_{12}\)H\(_{14}\)N\(_6\)] = 242.1280).

Anal. Calcd for C\(_{12}\)H\(_{14}\)N\(_6\): C, 59.49; H, 5.82; N, 34.69. Found: C, 59.55; H, 5.70; N, 34.78.

The sodium salt of 9b was treated with 3 molar equivalents of 1-iodobutane to give the 9-alkylated compound 39c (113 mg, 91%). No alkylation on the imidazole ring was detected.
6-(Imidazol-1-yl)-9-isopropylpurine (39d)

The sodium salt of 9b (95 mg, 0.5 mmol) was treated with 2-iodopropane (75 µL, 128 mg, 7.5 mmol) by general method 2. Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH₂Cl₂, 1:40) to give a solid (92 mg, 79%). Recrystallization (CH₂Cl₂/hexanes) gave 39d: mp 242.5-243.5 °C; UV (MeOH) max 212, 283, 292 nm (ε 23 800, 16 200, 12 100), min 235, 290, nm (ε 3200, 12 100); ¹H NMR (300 MHz, CDCl₃) δ 9.21 (s, 1H), 8.80 (s, 1H), 8.43 (s, 1H), 8.19 (s, 1H), 7.27 (s, 1H), 5.00 (sept, J = 6.8 Hz, 1H), 1.70 (d, J = 6.8 Hz, 6H); ¹³C NMR (75 MHz, CDCl₃) δ 153.6, 152.1, 145.9, 142.4, 137.9, 130.9, 123.1, 117.6, 48.0, 22.8; HRMS m/z 228.1132 (M⁺ [C₁₁H₁₂N₆] = 228.1123). Anal. Calcd for C₁₁H₁₂N₆: C, 57.88; H, 5.30; N, 36.82. Found: C, 58.00; H, 5.12; N, 37.03.

Compound 9b (98 mg, 0.5 mmol) was treated overnight with 2-iodopropane (150 µL, 266 mg, 1.5 mmol) by general method 3 (reaction complete, TLC). Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH₂Cl₂, 1:30) to give 39d (102 mg, 91%).

9-Cyclopentyl-6-(imidazol-1-yl)purine (39e)

The sodium salt of 9b (98 mg, 0.5 mmol) was treated with iodocyclopentane (290 µL, 492 mg, 2.5 mmol) by general method 2. Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH₂Cl₂, 1:40) to give a solid (0.123 g, 92%). Recrystallization (CH₂Cl₂/hexanes) gave 39e: mp 117.5-119 °C; UV (MeOH) max 283, 293 nm (ε 16 500, 12 300), min 240, 291 nm (ε 3800, 11 700); ¹H NMR (300 MHz, CDCl₃) δ 9.21 (s, 1H), 8.80 (d, J = 1.5 Hz, 1H), 8.43 (s, 1H), 8.17 (s, 1H), 7.28 (s, 1H), 5.07 (quint, J = 7.1 Hz, 1H), 2.41–2.35 (m, 2H), 1.86–2.13 (m, 6H); ¹³C NMR (75 MHz,
CDCl₃ δ 154.0, 152.1, 145.9, 143.0, 137.9, 130.9, 123.1, 117.6, 56.8, 32.9, 24.1; HRMS m/z 254.1291 (M⁺ [C₁₃H₁₄N₆] = 254.1280). Anal. Calcd for C₁₃H₁₄N₆: C, 61.40; H, 5.55; N, 33.05. Found: C, 61.21; H, 5.32; N, 33.13.

Compound 9b (98 mg, 0.5 mmol) was treated with iodocyclopentane (174 µL, 295 mg, 1.5 mmol) by general method 3. Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH₂Cl₂, 1:30) to give 39e (123 mg, 98%).

**9-Benzyl-6-(imidazol-1-yl)purine (39f)**

BnI was prepared from BnCl (0.086 mL, 0.75 mmol) and NaI (0.25 g, 1.5 mmol) in CH₃CN (2 mL) as previously described.

The sodium salt of 9b (98 mg, 0.5 mmol) was treated with the solution of BnI in CH₃CN by general method 2. Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH₂Cl₂, 1:40) to give a solid (133 mg, 95%). Recrystallization (CH₂Cl₂/hexanes) gave 39f: mp 195.5-196.5 °C; UV (MeOH) max 282, 292 nm (ε 16 800, 12 700), min 238, 290 nm (ε 3600, 12 300); ¹H NMR (300 MHz, CDCl₃) δ 9.21 (s, 1H), 8.86 (d, J = 1.2 Hz, 1H), 8.85 (s, 1H), 8.11 (s, 1H), 7.29–7.43 (m, 6H); 5.52 (s, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 154.0, 152.7, 146.0, 144.5, 137.9, 135.0, 131.0, 129.5, 129.1, 128.2, 122.6, 117.6, 47.9; HRMS m/z 276.1122 (M⁺ [C₁₅H₁₂N₆] = 276.1123). Anal. Calcd for C₁₅H₁₂N₆: C, 65.21; H, 4.38; N, 30.42. Found: C, 65.22; H, 4.31; N, 30.66.

**3-Benzyl-1-(9-benzylpurin-6-yl)imidazolium iodide (40)**

The sodium salt of 9b (93 mg, 0.5 mmol) was treated with a solution of BnI [prepared from BnCl (0.17 mL, 0.19 g, 1.5 mmol)] in CH₃CN by general method 2. Volatiles were evaporated in vacuo, and the residue was chromatographed
(MeOH/CH₂Cl₂, 1:40) to give 9-benzyl-6-(imidazol-1-yl)purine (20 mg, 14%) and 40 (179 mg, 73%): ¹H NMR (300 MHz, CDCl₃) δ 10.57–10.59 (m, 1H), 8.76 (s, 1H), 8.66 (s, 1H), 8.60–8.61 (m, 1H), 8.06–8.08 (m, 1H), 7.64–7.69 (m, 2H), 7.24–7.41 (m, 8H), 6.01 (s, 2H), 5.57 (s, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 154.8, 151.9, 147.9, 141.5, 135.6, 134.7, 132.5, 129.8, 129.5, 129.2, 128.9, 128.5, 124.4, 122.8, 120.0, 54.4, 48.1.

The bisbenzylated product 40 was treated with NaOMe/MeOH (1 M). Volatiles were evaporated in vacuo, and the residue was chromatographed (to remove NaOMe). The derived mixture was extracted with D₂O. The soluble component was N-benzylimidazole (contaminated with 9-benzyl-6-methoxypurine): ¹H NMR (300 MHz, D₂O) δ 7.63 (s, 1 H), 7.11–7.24 (m, 5 H), 6.98 (s, 1H), 6.88 (s, 1H), 5.06 (s, 2H); LRMS m/z 159 (MH⁺ [C₁₀H₁₁N₂] = 159). The residue was sublimed, and the condensed component was 1-(9-benzylpurin-6-yl)-3-benzyl-2-methoxy-2,3-dihydroimidazole: ¹H NMR (300 MHz, Acetone-d₆) δ 8.53 (s, 1H), 8.33 (s, 1H), 7.68 (s, 1H), 7.30–7.42 (m, 10H), 7.11 (s, 1H), 6.95 (s, 1H), 5.56 (s, 2H), 5.28 (s, 2H), 4.15 (s, 3H). The residue was mainly 9-benzyl-6-methoxypurine: ¹H NMR (300 MHz, Acetone-d₆) δ 8.52 (s, 1H), 8.34 (s, 1H), 7.31–7.44 (m, 5H), 5.56 (s, 2H), 4.15 (s, 3H); LRMS m/z 240 (M⁺ [C₁₃H₁₂N₄O] = 240).

9-Alkyl-2-amino-6-(imidazol-1-yl)purine (46a-d)

General method 4. A mixture of 34 (0.1 g, 0.5 mmol) in DMF (5 mL) was treated with sodium hydride (25 mg, 60% w/w suspension, 0.6 mmol), and the suspension was stirred at ambient temperature under N₂ for 1 h. The respective alkyl iodide was added, and the resulting mixture was stirred until the alkylation was completed as indicated by TLC.
General method 5. To a suspension of 34 (0.1 g, 0.5 mmol) and K₂CO₃ (0.23 g, 1.5 mmol) in DMF (5 mL) was added the respective iodoalkane. The mixture was stirred overnight.

2-Amino-9-butyl-6-(imidazol-1-yl)purine (46a-1)

Compound 34 (0.1 g, 0.5 mmol) was treated with 1-iodobutane (0.25 mL, 0.404 g, 2.20 mmol) by general method 5. The reaction was complete in 28 h. Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH₂Cl₂, 1:60) to give 46a-1 (103 mg, 81%) as the only product: mp 156.5-157 °C; UV (MeOH) max 227, 321 nm (ε 33 800, 9000), min 281 nm (ε 1400); ¹H NMR (500 MHz, DMSO-d₆) δ 8.92 (s, 1H), 8.24–8.23 (m, 1H), 7.19–7.18 (m, 1H), 6.80 (s, 2H), 4.08 (t, J = 7.3 Hz, 2H), 1.78 (quint, J = 7.3 Hz, 2H), 1.27 (sext, J = 7.3 Hz, 2H), 0.90 (s, J = 7.3 Hz, 3H); ¹³C NMR (125 MHz, DMSO-d₆) δ 160.6, 156.5, 145.6, 143.8, 137.2, 130.6, 117.7, 115.7, 43.3, 31.8, 20.0, 14.1; HRMS m/z 257.1378 (M⁺ [C₁₂H₁₅N₇] = 257.1389); Anal. Calcd for C₁₂H₁₅N₇: C, 56.02; H, 5.88; N, 38.11. Found: C, 55.82; H, 6.01; N, 37.88.

9-Butyl-2-butylamino-6-(imidazol-1-yl)purine (46a-2)

The sodium salt of 34 (0.1 g, 0.5 mmol) was treated with 1-iodobutane (0.25 mL, 0.404 g, 2.20 mmol) by general method 4. The reaction was complete in 5.5 h to give two products. Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH₂Cl₂, 1:96 → 1:60) to give 46a-1 (80 mg, 63%) and 46a-2 (12 mg, 7%). 46a-2: mp 134.5-135 °C; UV (MeOH) max 232, 333 nm (ε 35 400, 7600), min 212, 287 nm (ε 13 600, 3200); ¹H NMR (500 MHz, CDCl₃) δ 9.08 (s, 1H), 8.33 (s, 1H), 7.72 (s, 1H), 7.22 (s, 1H), 5.13 (s, 1H), 4.13 (t, J = 7.3 Hz, 2H), 3.50 (q, J = 7.0 Hz, 2H), 1.88 (quint, J = 7.3 Hz, 2H), 1.67 (quint, J = 7.3 Hz, 2H), 1.47 (sext, J = 7.3 Hz, 2H), 1.40 (sext, J =
7.3 Hz, 2H), 0.98–1.01 (m, 6 H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 159.4, 156.2, 146.1, 141.4, 137.7, 130.4, 117.6, 116.4, 43.4, 41.9, 31.99, 31.97, 20.4, 20.1, 14.1, 13.8; HRMS m/z 313.2007 (M$^+$ [C$_{16}$H$_{23}$N$_7$] = 313.2015); Anal. Calcd for C$_{16}$H$_{23}$N$_7$: C, 61.32; H, 7.40; N, 31.28. Found: C, 61.16; H, 7.46; N, 31.14.

2-Amino-9-benzyl-$6$-(imidazol-$1$-yl)purine (46b)

Compound 34 (0.1 g, 0.5 mmol) was treated with BnI solution in CH$_3$CN (0.3 M, 2.5 mL, 0.75 mmol) by general method 5 (reaction incomplete, TLC). Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH$_2$Cl$_2$, 1:60) to give 46b (99 mg, 69%) as the only product: mp 219.5-221 °C; UV (MeOH) max 227, 321 nm ($\varepsilon$ 31 300, 9400), min 213, 282 nm ($\varepsilon$ 22 600, 1700); $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 8.93 (s, 1H), 8.32 (s, 1H), 8.24 (s, 1H), 7.28–7.37 (m, 5H), 7.19 (s, 1H), 6.84 (s, 2H), 5.34 (s, 2 H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 160.8, 156.5, 145.8, 143.7, 137.5, 137.3, 130.6, 129.4, 128.4, 127.9, 117.8, 115.6, 46.6; HRMS m/z 291.1234 (M$^+$ [C$_{15}$H$_{13}$N$_7$] = 291.1232); Anal. Calcd for C$_{15}$H$_{13}$N$_7$: C, 61.84; H, 4.50; N, 33.66. Found: C, 61.68; H, 4.44; N, 33.75.

The reaction was repeated by general method 4. Minor byproducts were detected (TLC). The starting material disappeared in 5.5 h. Volatiles were evaporated, and the residue was chromatographed (MeOH/CH$_2$Cl$_2$, 1:30) to give 46b (111 mg, 77%).

2-Amino-9-cyclopentyl-$6$-(imidazol-$1$-yl)purine (46c)

Compound 34 (0.11 g, 0.5 mmol) was treated with iodocyclopentane (0.30 mL, 0.509 g, 2.59 mmol) by general method 5. Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH$_2$Cl$_2$, 1:30) to give 46c (142 mg, 99%) as the only product: mp 183.5-184.5 °C; UV (MeOH) max 228, 320 nm ($\varepsilon$ 31 900, 8500), min
210, 281 nm (ε 12 800, 1800); ¹H NMR (500 MHz, CDCl₃) δ 9.08 (s, 1H), 8.32 (s, 1H), 7.84 (s, 1H), 7.22 (s, 1H), 4.85 (quint, J = 7.3 Hz, 1H), 2.27–2.32 (m, 2H), 1.92–2.03 (m, 4H), 1.79–1.87 (m, 2H); ¹³C NMR (125 MHz, CDCl₃) δ 159.2, 156.0, 146.3, 140.2, 137.7, 130.5, 117.6, 117.5, 55.9, 32.8, 24.1; HRMS m/z 269.1383 (M⁺ [C₁₃H₁₅N₇] = 269.1389); Anal. Calcd for C₁₃H₁₅N₇: C, 57.98; H, 5.61; N, 36.41. Found: C, 57.91; H, 5.87; N, 36.28.

The reaction was repeated by general method 4. Minor byproducts were detected (reaction incomplete, TLC). The reaction mixture was diluted with CH₂Cl₂ (50 mL), washed (H₂O) and dried (Na₂SO₄). Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH₂Cl₂, 1:30) to give 46c (80 mg, 58%).

**2-Amino-9-acetoxyethoxymethyl-6-(imidazol-1-yl)purine (46d-1)**

The sodium salt of 34 (0.10 g, 0.5 mmol) was treated with acetoxyethoxymethyl bromide (118 mg, 0.6 mmol) by general method 4 for 18.5 h (reaction almost complete, TLC). Volatiles were evaporated in vacuo, and the residue was chromographed (MeOH/CH₂Cl₂, 1:40) to give 46d-1 (88 mg, 56%): mp 172-173.5 ºC; UV (MeOH) max 224, 320 nm (ε 29 700, 9000), min 281 nm (ε 2000); ¹H NMR (500 MHz, DMSO-d₆) δ 8.93 (s, 1H), 8.36 (s, 1H), 8.25 (s, 1H), 7.20 (s, 1H), 6.89 (s, 2H), 5.53 (s, 2H), 4.09 (t, J = 4.6 Hz, 2H), 3.74 (t, J = 4.6 Hz, 2H), 1.94 (s, 3H); ¹³C NMR (125 MHz, DMSO-d₆) δ 170.9, 160.9, 156.7, 145.8, 143.8, 137.2, 130.75, 117.8, 115.5, 72.7, 67.5, 63.5, 21.2; HRMS m/z 340.1147 (MNa⁺ [C₁₃H₁₅N₇O₃Na] = 340.1134); Anal. Calcd for C₁₃H₁₅N₇O₃: C, 49.21; H, 4.76; N, 30.90. Found: C, 49.28; H, 5.00; N, 30.72.

Alkylation of 34 by general method 5 with excess alkylation reagent (0.28 g, 1.42 mmol) gave two bisalkylated products (reaction complete, TLC), and with additional
alkylating reagent (0.121g, 0.61 mmol) gave mainly 46d-1 with minor byproducts (reaction was incomplete, TLC).

2-Acetamido-9-acetoxyethoxymethyl-6-(imidazol-1-yl)purine (46d-2)

The sodium salt of 29 (0.121 g, 0.5 mmol) was treated with acetoxyethoxymethyl bromide (118 mg, 0.6 mmol) by general method 4. The mixture was stirred for 30 min, and additional acetoxyethoxymethyl bromide (125 mg, 0.63 mmol) was added. The mixture was stirred for 9 h (reaction almost complete with some bisalkylated products, TLC). Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH$_2$Cl$_2$, 1:30) to give 46d-2 (56 mg) and a mixture including a bisalkylated product (18 mg, 7%) and 46d-2 (38 mg, 53% total): mp 178-178.5 °C; UV (MeOH) max 235, 298 nm (ε 34 000, 12 100), min 211, 277 nm (ε 14 300, 6800); $^1$H NMR (500 MHz, DMSO-$d_6$) δ 10.76 (s, 1H), 8.98 (‘t’, $J$ = 1.1 Hz, 1H), 8.72 (d, $J$ = 1.2 Hz, 1H), 8.31 (‘q’, $J$ = 1.2 Hz, 1H), 7.26 (‘t’, $J$ = 1.1 Hz, 1H), 5.66 (s, 2H), 4.09 (dd, $J$ = 4.8, 3.6 Hz, 2H), 3.79 (dd, $J$ = 4.8, 3.6 Hz, 2H), 2.28 (s, 3H), 1.93 (s, 3H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 170.9, 169.0, 154.8, 152.6, 145.9, 144.6, 136.6, 130.4, 118.0, 117.2, 72.4, 67.2, 62.6, 24.7, 20.4; HRMS m/z 382.1252 (MNa$^+$ [C$_{15}$H$_{17}$N$_7$O$_4$Na] = 382.1240); Anal. Calcd for C$_{15}$H$_{17}$N$_7$O$_4$: C, 50.14; H, 4.77; N, 27.29. Found: C, 50.32; H, 5.00; N, 27.29. 2-Acetamido-2-N,9-diacetoxyethoxymethyl-6-(imidazol-1-yl)purine: $^1$H NMR (500 MHz, DMSO-$d_6$) δ 9.07 (d, $J$ = 1.0 Hz, 1H), 8.84 (s, 1H), 8.42 (t, $J$ = 1.3 Hz, 1H), 7.27 (t, $J$ = 1.0 Hz, 1H), 5.72 (s, 2H), 5.52 (s, 2H), 4.08 (t, $J$ = 4.8 Hz, 2H), 4.04 (t, $J$ = 4.8 Hz, 2H), 3.77 (t, $J$ = 4.8 Hz, 2H), 3.70 (t, $J$ = 4.8 Hz, 2H), 2.37 (s, 3H), 1.93 (s, 3H), 1.92 (s, 3H).
9-Alky-2-chloro-6-(imidazol-1-yl)purine (47a-c)

General method 6. A solution of 2-chloro-6-(imidazol-1-yl)purine (14) (110 mg, 0.5 mmol) in DMF (5 mL) was treated with sodium hydride (25 mg, 60 % w/w suspension, 0.6 mmol) and stirred at ambient temperature under N₂ for 1 h. The respective alkyl iodide was added to the solution, and the resulting mixture was stirred until the alkylation was completed as indicated by TLC.

9-Butyl-2-chloro-6-(imidazol-1-yl)purine (47a)

The sodium salt of 14 (110 mg, 0.5 mmol) was treated with 1-iodobutane (0.35 ml, 566 mg, 3.1 mmol) by general method 6 for 4 h (reaction complete, TLC). Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH₂Cl₂, 1:60) to give 47a (122 mg, 87%): mp 150-150.5 °C; UV (MeOH) max 220, 290, 300 nm (ε 29000, 15 600, 11 100); ¹H NMR (500 MHz, DMSO-d₆) δ 9.15 (t, J = 1.1 Hz, 1H), 8.35 (t, J = 1.4 Hz, 1H), 8.07 (s, 1H), 7.25 (dd, J = 1.5, 0.9 Hz, 1H), 4.29 (t, J = 7.4 Hz, 2H), 1.93 (quint, J = 7.5 Hz, 2H), 1.41 (sext, J = 7.3 Hz, 2H), 0.99 (t, J = 7.0 Hz, 3H); ¹³C NMR (125 MHz, DMSO-d₆) δ 155.4, 153.9, 146.5, 145.1, 138.0, 131.2, 121.6, 117.6, 44.4, 32.1, 20.1, 13.7; HRMS m/z 276.0889 (M⁺ [C₁₂H₁₃ClN₆] = 276.0890). Anal. Calcd for C₁₂H₁₃ClN₆: C, 52.08; H, 4.74; N, 30.37. Found: C, 51.96; H, 4.86; N, 30.12.

9-Benzyl-2-chloro-6-(imidazol-1-yl)purine (47b)

The sodium salt of 14 (110 mg, 0.5 mmol) was treated with a BnI/CH₃CN solution (0.3 M, 2.5 mL, 0.75 mmol) by general method 6 for 12 h (reaction complete, TLC). Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH₂Cl₂, 1:60) to give 47b (131 mg, 85%): mp 211.5-212 °C; UV (MeOH) max
217, 290, 300 nm (ε 33 500, 16 200, 13 300), min 240, 297 nm (ε 2700, 12 500); ¹H
NMR (500 MHz, DMSO-d₆) δ 9.01 (s, 1H), 8.88 (s, 1H), 8.33 (s, 1H), 7.30–7.39 (m, 5H), 7.26 (s, 1H), 5.53 (s, 2H); ¹³C NMR (125 MHz, DMSO-d₆) δ 155.7, 152.7, 148.3, 146.2, 137.7, 136.5, 131.5, 129.5, 128.8, 128.3, 121.9, 118.1, 47.6; HRMS m/z 310.0739 (M⁺ [C₁₅H₁₁ClN₆] = 310.0734). Anal. Calcd for C₁₅H₁₁ClN₆: C, 57.98; H, 3.57; N, 27.05. Found: C, 58.13; H, 3.70; N, 27.17.

2-Chloro-9-cyclopentyl-6-(imidazol-1-yl)purine (47c)

The sodium salt of 14 (110 mg, 0.5 mmol) was treated with iodoncyclopentane (0.3 ml, 509 mg, 2.6 mmol) by general method 4 for 7 days (reaction almost complete, TLC). Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH₂Cl₂, 1:60) to give 47c as a solid (121 mg, 84 %): mp 182-182.5 °C; UV (MeOH) max 221, 290, 301 nm (ε 30 800, 15 600, 12 700), min 242, 298 nm (ε 3600, 12 200); ¹H NMR (500 MHz, CDCl₃) δ 9.02 (s, 1H), 8.85 (s, 1H), 8.33 (s, 1H), 7.27 (s, 1H), 4.97 (quint, J = 7.3 Hz, 1H), 2.20–2.26 (m, 2H), 2.00–2.07 (m, 2H), 1.87–1.95 (m, 2H), 1.70–1.78 (m, 2H); ¹³C NMR (125 MHz, CDCl₃) δ 155.7, 152.2, 146.9, 146.0, 137.7, 131.5, 122.2, 118.1, 56.9, 32.6, 24.2; HRMS m/z 288.0888 (M⁺ [C₁₃H₁₃ClN₆] = 288.0890). Anal. Calcd for C₁₃H₁₃ClN₆: C, 54.08; H, 4.54; N, 29.11. Found: C, 54.30; H, 4.65; N, 29.14.

2-Chloro-7/9-ethyl-6-(4,5-diphenylimidazol-1-yl)purine (50/51)

A mixture of 2-chloro-6-(4,5-diphenylimidazol-1-yl)purine (54 mg, 0.13 mmol) and sodium hydride (8.6 mg, 60 % w/w suspension, 0.21 mmol) was stirred in DMF at ambient temperature under N₂ for 1 h. The sodium salt was treated with iodoethane (0.05
mL, 99 mg, 0.62 mmol) for 3 h (reaction almost complete, TLC). The reaction mixture contained two products. Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH₂Cl₂, 1:30) to give the two compounds (N9/N7, 5:1). 2-Chloro-9-ethyl-6-(4,5-diphenylimidazol-1-yl)purine (50): UV (MeOH) max 278 nm (ε 16300), min 268 nm (ε 15 700); ¹H NMR (500 MHz, DMSO-d₆) δ 8.86 (s, 1H), 8.78 (s, 1H), 7.20–7.48 (m, 10H), 4.28 (q, J = 7.3 Hz, 2H), 1.45 (t, J = 7.3 Hz, 3H); ¹³C NMR (125 MHz, DMSO-d₆) δ 154.7, 150.8, 147.6, 145.9, 139.0, 138.8, 133.4, 130.6, 130.4, 128.23, 128.16, 128.1, 127.2, 127.0, 126.8, 124.1, 40.0, 14.6; NOESY cross peaks: (CH₂, H8), (CH₂, CH₃), (CH₂, Ph), (CH₃, H8) and (CH₃, Ph); HRMS m/z 400.1207 (M⁺ [C₂₂H₁₇ClN₆] = 400.1203). Anal. Calcd for C₂₂H₁₇ClN₆: C, 65.92; H, 4.27; N, 20.96. Found: C66.00; H, 4.50; N, 20.83.

2-Chloro-7-ethyl-6-(4,5-diphenylimidazol-1-yl)purine (51): ¹H NMR (500 MHz, DMSO-d₆) δ 8.95 (s, 1H), 8.46 (s, 1H), 7.20–7.52 (m, 10H), 4.05 (q, J = 7.3 Hz, 2H), 1.20 (t, J = 7.3 Hz, 3H); NOE difference: CH₂ was irradiated, NOE effects with H8 (1.9%), H₂’ (imidazolyl) (3.6%), Ph (2.9%) and CH₃ (4.8%); NOESY cross peaks: (CH₂, H8), [CH₂, H₂’ (imidazolyl)], (CH₂, CH₃), (CH₂, Ph), (CH₃, H8), [CH₃, H₂’ (imidazolyl)] and (CH₃, Ph); ¹³C NMR (125 MHz, DMSO-d₆) δ 166.1, 153.6, 151.7, 147.6, 142.1, 139.3, 139.0, 134.2, 130.8, 129.5, 129.2, 129.0, 127.9, 127.5, 120.6, 42.9, 15.8; HRMS m/z 400.1212 (M⁺ [C₂₂H₁₇ClN₆ = 400.1203]).

6-(2-Butylimidazol-1-yl)-2-chloro-9-ethylpurine (52)

A mixture of 6-(2-butylimidazol-1-yl)-2-chloropurine (50 mg, 0.18 mmol) and sodium hydride (11.2 mg, 60% w/w suspension, 0.27 mmol) in DMF was stirred at ambient temperature under N₂ for 1 h. The sodium salt was treated with iodoethane (0.09
mL, 179 mg, 1.12 mmol) for 4 h to give a single product (reaction complete, TLC).

Volatiles were evaporated in vacuo, and the residue was chromatographed
(MeOH/CH₂Cl₂, 1:30) to give 52 (quantitative): mp 104.5-105 °C; UV (MeOH) max 218,
289 nm (ε 23 700, 13 300), min 240 nm (ε 1500); ¹H NMR (500 MHz, DMSO-d₆) δ 8.77
(d, J = 1.0 Hz, 1H), 8.45 (t, J = 1.3 Hz, 1H), 7.07 (t, J = 1.5 Hz, 1H), 4.31 (q, J = 7.3 Hz,
2H), 3.12 (t, J = 7.3 Hz, 3H), 1.68 (quint, J = 7.3 Hz, 2H), 1.47 (t, J = 7.3 Hz, 2H), 4.31
(sext, J = 7.3 Hz, 2H), 0.91 (t, J = 7.3 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 154.8,
150.9, 149.6, 146.8, 146.7, 128.2, 122.3, 120.3, 38.9, 29.5, 29.4, 21.9, 14.7, 13.6; HRMS
m/z 327.1105 (MNa⁺ [C₁₄H₁₇ClN₆Na = 327.1101]). Anal. Calcd for C₁₄H₁₇ClN₆: C,
55.17; H, 5.62; N, 27.57. Found: C, 55.01; H, 5.72; N, 27.57.
5. References and Notes


   (b) Allart, B.; Busson, R.; Rozenski, J.; Van Aerschot, A.; Herdewijn, P.


17. (a) Kamaike, K.; Takahashi, M.; Utsugi, K.; Tomizuka, K.; Ishido, Y.


38. Hakimelahi, G. H.; Ly, T. W.; Moosavi-Movahedi, A. A.; Jain, M. L.; Zakerinia, 
   M.; Davari, H.; Mei, H.-C.; Sambaiah, T.; Moshfegh, A. A.; Hakimelahi, S. J. 

   2330.


41. Bressi, J.; Choe, J.; Hough, M. T.; Buckner, F. S.; Voorhis, W. C. V.; Verlinde, C. 


46. Kozai, S.; Yorikane, A.; Maruyama, T. *Nucleosides Nucleotides Nucleic Acids* 
   **2001**, *20*, 1523-1531.

47. Hrebabecky, H.; Farkas, J. In *Nucleic Acid Chemistry*; Townsend, L. B., Tipson, 


55. The twisted conformer of 2-amino-4-(2-chloro-4,5-dimethoxyphenyl)-1,3-thiazole was reported to be stabilized by intermolecular hydrogen bonding in the solid state. (a) Bernes, S.; Berros, M.; Rodriguez de Barbarin, C.; Sanchez-Viesca, F. *Acta Cryst.* **2002**, *C58*, o151-o153. (b) Rodriguez de Barbarin, C.; Bernes, S.; Sanchez-Viesca, F.; Berros, M. *Acta Cryst.* **2003**, *C59*, o360-o362.


Chapter 4

Glycosylation of 6-Heteroarylpurines, Regiospecificity and Stereoselectivity

1. Introduction

The major difficulties with the synthesis of 2’-deoxynucleosides lie in simultaneously achieving regio- and stereocontrol of glycosylation. The general methods for synthesis of nucleosides were summarized in Chapter 1. One widely used method involves S_N2 substitution of 2-deoxy-3,5-di-O-(p-toluoyl)-α-D-erythro-pentofuranosyl chloride by the sodium salt of bases. However, the stereoselectivity varies with heterocyclic bases and reaction conditions (e.g., solvents, temperature, etc.)

Solvent polarity strongly affects the stereoselectivity of glycosylation.\(^2\)\(^-\)\(^4\) Nonpolar solvents decrease the anomerization and decomposition of the chlorosugar, but poorly dissolve the sodium salts.\(^2\) Kawakami et al. used moderately polar solvents such as acetone as the reaction medium and obtained moderate yields.\(^3\) Ugarkar et al. reported stereoselective glycosylation of 7-deazapurines with 5-deoxy-2,3-O-isopropylidene-α-D-ribofuranosyl chloride in toluene with tris[2-(methoxyethoxy)ethyl]amine (TDA-1) as a phase-transfer catalyst to improve the solubility of the sodium salt.

Solvation plays an important role in nucleophilic substitution reactions. Brauman et al.\(^5\) reported measurements of S_N2 reactions of chloride ion with methyl- and t-butyl-substituted chloroacetonitriles using Fourier transform–ion cyclotron resonance spectrometry and the results of Monte Carlo simulations on related thermoneutral reactions of alkyl chlorides. The difference in the free energy of activation for the two
reactions in the gas phase is much smaller than that in solution. The distinct contributions of the steric effects and solvation to the differences in the free energy of activation were estimated to be 1.6 kcal/mol and 4 kcal/mol, respectively. The differences in solvation were attributed to the greater increase in the free energy ($\Delta G > 0$) of desolvation for the larger neopentyl system relative to the methyl chloride system.

The attractive solute-solvent interactions can be divided into two types: (1) nonspecific dipolarity/polarizability and (2) specific hydrogen-bond complex formation. The scale of dipolarity/polarizability ($\pi^*$) was obtained from linear solvent shifts in $\pi \rightarrow \pi^*$ ultraviolet transition energies, and the potency of hydrogen-bond complex formation was measured by hydrogen-bond-donor (HBD) acidity ($\alpha$) and hydrogen-bond-acceptor (HBA) basicity ($\beta$). These solvatochromic parameters of some related solvents are given in Table 1. A polarization correction term might be needed, and dipolarity/polarizability might be better measured by dielectric constants. The dielectric constants of some solvents are given in Table 2.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$\pi^*$</th>
<th>$\alpha$ (HBD)</th>
<th>$\beta$ (HBA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>toluene</td>
<td>0.54</td>
<td>0.00</td>
<td>0.11</td>
</tr>
<tr>
<td>$\text{CH}_2\text{Cl}_2$</td>
<td>0.82</td>
<td>0.30</td>
<td>~ 0.05</td>
</tr>
<tr>
<td>$\text{CH}_3\text{CN}$</td>
<td>0.75</td>
<td>0.19</td>
<td>0.35</td>
</tr>
<tr>
<td>DMF</td>
<td>0.88</td>
<td>0.00</td>
<td>0.69</td>
</tr>
<tr>
<td>THF</td>
<td>0.58</td>
<td>0.00</td>
<td>0.55</td>
</tr>
</tbody>
</table>

**Table 1. Solvatochromic parameters of some related solvents**
Table 2. Dielectric constants of some solvents

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Dielectric constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMF</td>
<td>38.25</td>
</tr>
<tr>
<td>CH$_3$CN</td>
<td>36.64</td>
</tr>
<tr>
<td>Acetone</td>
<td>21.01</td>
</tr>
<tr>
<td>CH$_2$Cl$_2$</td>
<td>8.93</td>
</tr>
<tr>
<td>THF</td>
<td>7.52</td>
</tr>
<tr>
<td>CHCl$_3$</td>
<td>4.81</td>
</tr>
<tr>
<td>Toluene</td>
<td>2.38</td>
</tr>
<tr>
<td>Benzene</td>
<td>2.28</td>
</tr>
</tbody>
</table>

The solvent effect on rate constant can be written as:

$$
\ln k = \ln k_0 + s(\pi^* + d\delta) + a\alpha + b\beta
$$

where $\delta$ is a polarization correction term. It was observed that the rates of bimolecular substitution reactions of methyl iodide with anionic nucleophiles depended on solvent HBD acidity ($\alpha$). The reaction rate constants decreased in order of increasing HBA solvation of the anions, which suggested that HBA desolvation of nucleophiles contributed greatly to the increase in the free energy of activation for the $S_N$2 pathway.

Lewis acid–catalyzed glycosylation usually occurs via an ionization mechanism ($S_N$1). The ionization step is rate determining, and the transition state resembles the oxocarbenium cation. Both steric and electronic effects affect the reaction rate, and the most important electronic effects are stabilization of the carbocation and the ability of the
leaving group to accept the electron pair. The trichloroacetimidate method used in Chapter 2 is an application of increasing the ability of the leaving group to accept the electron pair.

Ionization of sugar acetates under Lewis acid conditions results in charge separation in the transition state. Solvation influences the transition state more than the reactants. Solvents of higher dipolarity/polarizability (\(\pi^*\)), HBD acidity (\(\alpha\)) and HBA basicity (\(\beta\)) lower the energy of the transition state more than that of the reactants, and thus lower the activation energy (Figure 1).

Stereoselective glycosylation of 2-deoxysugar derivatives via the sodium salt method, however, usually requires the direct displacement mechanism (S\(_{N2}\)). In the transition state, the carbon p-orbital of the reaction center interacts with two equivalent occupied orbitals, one from the leaving group and the other from the nucleophile. The interactions of these orbitals give rise to three MOs, and the HOMO of the transition state is \(\pi\) in character (Figure 1). The solvent of higher dipolarity/polarizability (\(\pi^*\)), HBD acidity (\(\alpha\)) and HBA basicity (\(\beta\)) lowers the energy of the reactants more than that of the transition state, and thus increases the activation energy. Therefore, solvents of lower dipolarity/polarizability (\(\pi^*\)), HBD acidity (\(\alpha\)) and HBA basicity (\(\beta\)) should increase the selectivity for the S\(_{N2}\) pathway.

However, solubilities of the sodium salts of simple purine derivatives in nonpolar solvents are too low for practical reaction rates. It is well known that the solubility of a solute depends on solute-solute, solute-solvent and solvent-solvent interactions. In a specific solvent, the structure of the solute is the main factor influencing its solubility. Gani et al.\(^{10}\) and Meylan et al.\(^{11}\) have recently reported estimations of octanol/water
partition coefficient (log $K_{OW}$) for a wide range of chemical substances using group-contribution (GC) methods. The derived parameters indicate that nonpolar functions contribute positively to log $K_{OW}$. Therefore, by introducing nonpolar functions into a solute molecule, its solubility in less polar solvents can be enhanced.

Stereospecific formation of the $\beta$-isomer of nucleosides requires exclusive $S_N$2 displacement and the exclusion of anomerization of the $\alpha$ chlorosugar. With our achievement of regiospecificity for alkylation of modified purines, reaction conditions were evaluated for regiospecific and highly stereoselective 9-glycosylation of modified purines. The core of this strategy was to tune the reactions by changing solvent properties and modification of purine structures to minimize $S_N$1 pathways.

**Figure1. Potential energy diagrams for glycosylation**
2. Results and Discussion

2.1. Attempts at Glycosylation of Ribofuranose Derivatives Using 6-(Imidazol-1-yl)purines

Glycosylations of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose with 6-(imidazol-1-yl)purines were tried first for their simplicity. Glycosylation of 1-O-acetyl-2,3,5-tri-O-benzoylribofuranose under Lewis acid conditions give only β-nucleosides due to the anchimeric assistance of the 2-O-benzoyl group.

2.1.1. Glycosylation of Ribofuranose Derivatives

6-(Imidazol-1-yl)purines were persilylated in DCE with BSA at 80 °C. Volatiles were evaporated in vacuo, and the persilylated bases were coupled with 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose in toluene at 80 °C with TMSOTf as catalyst. The results are summarized in Scheme 1. Excess TMSOTf was needed to drive the reaction to completion. The coupling reaction per se was good for 6-(imidazol-1-yl)purine and 2-amino-6-(imidazol-1-yl)purine, and gave only N9 regioisomers. However, the basic N3 atom of the imidazole ring was functionalized, and addition of water occurred during deprotonation with mild bases to give 6-(2-hydroxy-2,3-dihydroimidazol-1-yl)purine nucleosides. Further experiments indicated that N3 of the imidazole ring of 6-(imidazol-1-yl)-9-isopropylpurine can be further glycosylated to give 3d under Lewis acid conditions (Figure 2).

A coupling reaction using SnCl₄ in dried CH₃CN at ambient temperature was very successful with 6-(imidazol-1-yl)purine, and gave 9-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-6-(imidazol-1-yl)purine (3a) quantitatively. A coupling reaction with 2-
acetamido-6-(imidazol-1-yl)purine using SnCl$_4$ gave the desired product in low yields plus hydrolyzed products. Elevation of the temperature to 65 °C did not improve the yield. A poor coupling with 2-amino-6-(imidazol-1-yl)purine was observed.

$$\text{N} \quad \text{N} \quad \text{N} \quad \text{N}$$

$$\text{N} \quad \text{N} \quad \text{N} \quad \text{N}$$

$$\text{R} \quad \text{R}$$

1. BSA, DCE 80 °C  
2. TMSOTf, 2 Tol. 80 °C  

SnCl$_4$, 2  
CH$_3$CN

<table>
<thead>
<tr>
<th>R</th>
<th>1a-c</th>
<th>3a-c</th>
<th>4a-c</th>
</tr>
</thead>
<tbody>
<tr>
<td>H (a)</td>
<td>(100%)</td>
<td>(11%) + 4 (35%) (r.t.)</td>
<td></td>
</tr>
<tr>
<td>NH$_2$ (b)</td>
<td>(83%)</td>
<td>(13%) (65 °C)</td>
<td></td>
</tr>
<tr>
<td>NHAc (c)</td>
<td>(70 %)</td>
<td>multi spots (TLC)</td>
<td></td>
</tr>
</tbody>
</table>

| 3d |

**Scheme 1. Glycosylation of 6-(imidazol-1-yl)purines with 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose under Lewis acid conditions**

$$\text{N} \quad \text{N} \quad \text{N} \quad \text{N}$$

$$\text{BzO} \quad \text{O} \quad \text{BzO}$$

$$\text{OAc} \quad \text{O} \quad \text{OAc}$$

$$\text{BzO} \quad \text{O} \quad \text{BzO}$$

$$\text{BzO} \quad \text{O} \quad \text{BzO}$$

$$\text{BzO} \quad \text{O} \quad \text{BzO}$$

$$\text{Ph}$$

| 3a-c |

**Figure 2. N3 glycosylation of the imidazole ring**
In conclusion, Vorbrüggen-type coupling reactions of 6-(imidazol-1-yl)purines under Lewis acid conditions were generally not very effective. An efficient method to scavenge the electrophiles formed in situ is needed.

Glycosylation of the sodium salt of 2-amino-6-(imidazol-1-yl)purine was tried with 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride. 1-O-Acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose was treated with HOAc/SOCl₂ in CH₂Cl₂ to give 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride as an anomeric mixture.¹³ Volatiles were evaporated, and the residue was used without further purification. Coupling of the sodium salt of 2-amino-6-(imidazol-1-yl)purine in DMF with this crude sugar chloride was incomplete and gave low yields (18%) with only one nucleoside separated (Scheme 2).

The failure of this reaction might result from the crude sugar chloride. Crystalline 2-deoxy-3,5-di-O-(p-toluoyl)-α-D-erythro-pentofuranosyl chloride can be prepared and stored in a sealed bottle at -20 °C without decomposition or anomerization for months, although it decomposes to a gray-colored powder if exposed to atmospheric humidity.¹⁴ Coupling of this sugar chloride with 6-(imidazol-1-yl)purines was tried next.

![Scheme 2. Glycosylation of 2-amino-6-(imidazol-1-yl)purine with crude 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride by the sodium salt method](image-url)
2.2. Glycosylation of a 2-Deoxy-\(\alpha\)-D-erythro-pentofuranose Derivative by the Sodium Salt Procedure

2.2.1. Quantitative \(^1\)H NMR

\(^1\)H NMR has been widely used for determinations of enantiomeric excess (ee) and distereomeric ratio (dr).\(^{15-17}\) Lewis et al.\(^{18}\) have studied the ground-state conformational equilibria of \textit{syn} and \textit{anti} \(N,N'\)-dimethyl-\(N,N'\)-di-1-naphthylurea protophanes using \(^1\)H NMR. The relative equilibrium concentrations were calculated based on iterative full line shape simulation. Prati et al.\(^{19}\) have developed (\(S\)-(+)\)-\(N\)-acetylphenylglycineboronic acid as a chiral derivatizing agent for enantiomeric excess (ee) determination of 1,2-diols. The reliability of determination methods of ee by \(^1\)H NMR was verified by comparison of the values obtained from diastereotopic proton signals with the known values of prepared samples. Harada et al.\(^{20}\) reported chiral auxiliaries for determination of ee by \(^1\)H NMR. Giner et al.\(^{21}\) and Kumar, et al.\(^{22}\) have also determined the ee of products using \(^1\)H NMR analysis with chiral reagents.

One significant advantage of \(^1\)H NMR is that the intensity of each signal is proportional to the number of nuclei giving the resonance. With proper control of the experimental conditions, the proportionality constant is the same for all resonances in a spectrum.\(^{23-27}\) Thus the relative concentrations of components can be obtained directly from the relative resonance intensities. However, NMR has the disadvantage of low sensitivity, although the sensitivity has increased dramatically over the past decade. To maintain the quantitative intensity relationships between resonance peaks, parameters such as rf pulse widths, relaxation delays, digital resolution and other acquisition and processing factors should be monitored. It was reported that quantitation accuracies of
1% can be attained for major components with proper consideration of these experimental conditions.25-27

To ensure this accuracy and reliability of relative intensities, differential saturation effects should be avoided with adequate signal-to-noise (S/N). Following a 90° pulse, magnetization in the xy plane decays by spin-spin relaxation, while it recovers along the z axis by spin-lattice relaxation. $M_{xy}$ decreases according to the equation (1):

$$M_{xy} = M_0 e^{-\frac{t}{T_{2}^*}}$$  \hspace{1cm} (1)

where $t$ is the time following the rf pulse and $1/T_{2}^*$ is the effective rate constant for FID decay, a function of the natural spin-spin relaxation time $T_2$ and the magnetic field inhomogeneity $\Delta B_0$. Magnetization returns to equilibrium along the z axis according to the equation (2):

$$M_z = M_0 (1 - e^{-\frac{t}{T_1}})$$  \hspace{1cm} (2)

where $T_1$ is the spin-lattice relaxation time. Generally $T_{2}^* < T_1$, $M_{xy}$ and the signal decreases to zero before $M_z$ recovers to $M_0$. Thus, acquiring data while pulsing rapidly relative to spin relaxation times leads to perturbation of the relative resonance intensities in the spectrum, and one strategy is to extend the recycle times to at least 5 $T_1$ of the slowest relaxing nuclei to ensure the full recovery of spins during pulses. This causes a tediously long experimental time, especially in the study of heteronuclei, although relaxation times can be reduced by introducing relaxation reagents. A longer pulse repetition time also causes lower S/N.

In this study, the relaxation delay (d1) was set to zero to shorten the experimental time. The deviation from relative intensities could be corrected by calibration. The Varian
500 MHz instrument was calibrated with a binary mixture (toluene/EtOAc) in CDCl$_3$ as shown (Figure 3). $T_1$ was fairly short for all methyl groups, and full recovery was nearly attained during the data acquiring period. The relative resonance intensities agree with the relative concentrations very well, in spite of a larger deviation observed for the ratio of a triplet (Me in EtOAc) to the singlet (Me in toluene) which might be caused by data processing factors and digital resolution.

Figure 3. Calibration of the Varian 500 MHz instrument (EtOAc/toluene)
Figure 4. Calibration of the VARIAN 500 MHz instrument with 9-(2-deoxy-3,5-di-O-(p-toluoyl)-α/β-D-erythro-pentofuranosyl)-6-(2-propylimidazol-1-yl)purines
Figure 5. Effects of relaxation delays on the accuracy of measured dr values of glycosylation products of 2-chloro-6-(2-isopropylimidazol-1-yl)purine

The Varian 500 MHz instrument was again calibrated by the H1’ resonances [a narrow doublet of doublets for α-anomer (δ ~6.71), and a wide doublet of doublets for β-anomer (δ ~6.63)] of mixtures of 9-(2-deoxy-3,5-di-O-(p-toluoyl)-α/β-D-erythrose-pentofuranosyl)-6-(2-propylimidazol-1-yl)purines with d1 = 0 s. The calibration curve presented a linear relation to give a proportionality constant (≠ 1) as shown in Figure 4. The deviation from unity of the experimental constant might be caused by data processing factors and/or digital resolution. T1 should be very short based on further experiments on the effects of relaxation delays. Increased relaxation delays did not affect the relative intensities much, but caused lower S/N (Figure 5).

Linear response of the instrument can ensure the reliability of the analysis of the effects of introduced lipophilic functions and solvents on the stereoselectivity of glycosylation based on 1H NMR data. The graph in Figure 4 suggested inaccuracy of
ratios higher than 20:1, which should depend on the concentration of the minor component and the sensitivity of the instrument.

2.2.2. Chemistry of 2-Deoxy-3,5-di-O-(p-toluoyl)-α-D-erythro-pentofuranosyl Chloride

2-Deoxy-3,5-di-O-(p-toluoyl)-α-D-erythro-pentofuranosyl chloride is a widely used precursor for the synthesis of 2’-deoxynucleosides. Kool et al. prepared the deoxynucleoside of 4-fluoro-6-methylbenzimidazole by glycosylation of the sodium salt with this sugar chloride in CH$_3$CN. Two β-regioisomers were formed in moderate combined yield. Seela et al. reported a synthesis of 8-aza-7-deaza-2’-deoxyadenosine by coupling the sodium salt in CH$_3$CN. A mixture of two β-regioisomers was obtained (56%). 7-Deazapurine was also coupled with the sugar chloride in CH$_3$CN to give the 2’-deoxynucleoside (65%).

2-Deoxy-3,5-di-O-(p-toluoyl)-α-D-erythro-pentofuranosyl chloride was prepared according to a reported procedure (Scheme 3). 2-Deoxy-D-erythro-pentose was converted into an anomeric mixture of methyl pentofuranosides, which was acylated with (p-toluoyl) chloride. The derived mixture was treated with AcCl/HOAc to give the sugar chloride, precipitated as a crystalline solid (53% for 3 steps).

Anomerization of the chloro sugar in CDCl$_3$ was studied by $^1$H NMR (Figure 6). $X_\alpha$ is the molar fraction of the α anomer in the anomeric mixture, which decreased with time. Hubbard et al. investigated the anomerization in various solvents using $^1$H NMR. It was observed that the β chlorosugar was formed faster in polar solvents like CH$_3$CN (70% in 2 h) than was formed in nonpolar solvents like CH$_2$Cl$_2$ (30% in 2 h) and CHCl$_3$ (20% in 2 h). No significant anomerization was observed in benzene. Therefore, fast
glycosylation in nonpolar solvents is required for high stereoselectivity. However, nonpolar solvents like toluene and benzene are poor media for glycosylations using the sodium salt method due to the low solubility of the sodium salts. One strategy to overcome this limitation is to increase the solubility of the sodium salt in a relatively nonpolar solvent by introduction of lipophilic functions, and to use solvent mixtures for the glycosylation reaction.

First, glycosylation of 6-(1,2,4-triazol-4-yl)purine and 6-(imidazol-1-yl)purine were studied.

![Scheme 3. Synthesis of 2-deoxy-3,5-di-O-(p-toluoyl)-α-D-erythro-pentofuranosyl Chloride](image)

**Figure 6. Anomerization of the α chlorosugar in CDCl$_3$ at ambient temperature**
2.2.3. Coupling of 6-(Imidazol-1-yl) and 6-(1,2,4-Triazol-4-yl)purine with 2-Deoxy-3,5-di-\(O\)-(p-toluoyl)-\(\alpha\)-D-\textit{erythro}-pentofuranosyl Chloride

6-(1,2,4-Triazol-4-yl)purine was prepared as previously described. Coupling of the sodium salt of 6-(1,2,4-triazol-4-yl)purine and 2-deoxy-3,5-di-\(O\)-(p-toluoyl)-\(\alpha\)-D-\textit{erythro}-pentofuranosyl chloride gave an anomeric mixture of N9 nucleosides as summarized in Scheme 4. The ratios given are the relative intensities of the \(^1\text{H} \text{NMR}\) resonances of H1'. The stereoselectivity was poor, with the best result obtained in a binary solvent mixture (DMF/\(\text{CH}_3\text{CN}\), 1:1) by treatment of the purine salt in DMF with the sugar chloride in \(\text{CH}_3\text{CN}\). The stereoselectivity of glycosylation in \(\text{CH}_3\text{CN}\) was even less than that in DMF in spite of the lower dielectric constant for \(\text{CH}_3\text{CN}\). The low stereoselectivity of glycosylation in DMF could be caused by its high dielectric constant, and DMF also could act as a moderate Lewis base. This could stabilize the oxocarbenium ion for \(S_N^1\) reactions including anomerization of the sugar chloride. \(\text{CH}_3\text{CN}\) is a much weaker Lewis base than DMF, but it is a better HBD.

6-(Imidazo1-1-yl)purine was prepared as previously described. Coupling of the sodium salt of 6-(imidazo1-1-yl)purine and 2-deoxy-3,5-di-\(O\)-(p-toluoyl)-\(\alpha\)-D-\textit{erythro}-pentofuranosyl chloride also gave anomeric mixtures of N9 nucleosides (Scheme 5). Solvents with a wider range of dielectric constants were used. Moderate stereoselectivity was obtained in \(\text{CH}_3\text{CN}/\text{toluene}\) (1:1) by treatment of the purine salt in \(\text{CH}_3\text{CN}\) with the sugar chloride in toluene. It seemed that the stereoselectivity increased with a decrease in solvent polarity, but further reduction in the polarity of solvents (DCM) resulted in very low conversion yields and poor stereoselectivity.
Scheme 4. Stereoselectivity for glycosylation of the sodium salt of 11

<table>
<thead>
<tr>
<th>Solvent 1/2</th>
<th>12/13</th>
<th>Yield%</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMF/DMF</td>
<td>1/1.1</td>
<td>58</td>
</tr>
<tr>
<td>DMF/DMF (5 mL)</td>
<td>2.5/1</td>
<td>54-83</td>
</tr>
<tr>
<td>+ CH₃CN (25 mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH₃CN/CH₃CN</td>
<td>1/1.62</td>
<td>40</td>
</tr>
</tbody>
</table>

Scheme 5. Stereoselectivity for glycosylation of the sodium salt of 1a

<table>
<thead>
<tr>
<th>Solvent 1/2</th>
<th>14/15</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMF/DMF</td>
<td>1/1.6</td>
</tr>
<tr>
<td>DMF/CH₃CN (5 mL) + DMF (5 mL)</td>
<td>1/1</td>
</tr>
<tr>
<td>CH₃CN/CH₃CN</td>
<td>1/1.7</td>
</tr>
<tr>
<td>CH₃CN/CH₃CN (5 mL) + Toluene (5 mL)</td>
<td>6.9/1</td>
</tr>
</tbody>
</table>

*Value in parenthesis is from the crude reaction mixture.
2.2.4. Improvements of Stereoselectivity of Glycosylation

The above results suggested that solvents of lower polarity with low HBA basicity (β) and HBD acidity (α) gave higher stereoselectivity for the formation of β nucleosides. Next, purine derivatives were further modified by introduction of lipophilic groups to increase their solubilities in moderately polar solvents. The imidazole ring has three sites capable of bearing alkyl groups. Alkyl chains were chosen to avoid disruption of the coplanar structures (Figure 19 in Chapter 3).

2.2.4.1. Synthesis of 6-(2-Alkylimidazol-1-yl)purines

2-Alkylimidazoles were prepared according to a reported procedure. Imidazole was protected to give 1-(N,N'-dimethylaminomethyl)imidazole (72%). Lithiation by treatment with butyllithium/hexanes in THF, alkylation, and subsequent acid-catalyzed hydrolysis of the protecting group gave the 2-alkylimidazoles in good yields (Scheme 6). Bis-alkylation at C2 and N3 occurred, which caused difficulty in separations and lowered the yields.

Scheme 6. Synthesis of 2-alkylimidazoles

The 6-oxo function of protected inosine was displaced with 2-alkylimidazoles (R = propyl, hexyl, dodecyl) as previously described (Scheme 7). 2’,3’,5’-Tri-O-
acetylinosine was treated with a 2-alkylimidazole, Ph3P, I2, EtN(i-Pr)2 in toluene at 95 °C overnight to give 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-6-(2-alkylimidazol-1-yl)purine. Deglycosylation in AcCl/HOAc gave the 6-(2-alkylimidazol-1-yl)purines in moderate to good yields for the three steps (R = propyl, 72%; hexyl, 63%; dodecyl, 54%).

<table>
<thead>
<tr>
<th>R</th>
<th>Yield% (for 2 steps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexyl (a)</td>
<td>63</td>
</tr>
<tr>
<td>Dodecyl (b)</td>
<td>54</td>
</tr>
<tr>
<td>Propyl (c)</td>
<td>72</td>
</tr>
</tbody>
</table>

Scheme 7. Synthesis of 6-(2-alkylimidazol-1-yl)purines

2.2.4.2. Coupling of 6-(2-Alkylimidazol-1-yl)purines with the Chlorosugar

6-(2-Alkylimidazol-1-yl)purines were then coupled with 2-deoxy-3,5-di-O-(p-toluoyl)-α-D-erythro-pentofuranosyl choride by the sodium salt method in various solvents (Schemes 8-10). Significant effects of solvents and alkyl chains on the stereoselectivity were observed.

A binary mixture of solvents (CH3CN/toluene or CH3CN/CH2Cl2) usually gave the highest stereoselectivity by treatment of the purine salt in CH3CN with the sugar
chloride in toluene or CH₂Cl₂. For 6-(2-propylimidazol-1-yl)purine, the stereoselectivity for glycosylation varied from an anomeric ratio (α/β) of 1.4:1 in DMF to 1:33.7 in CH₃CN/toluene (1:1, v/v) and 0:1 in CH₃CN/CH₂Cl₂ (1:1, v/v). The yield for the coupling reaction in a neat nonpolar solvent (toluene) was extremely low. The solvent effect was much less significant for 6-(2-hexylimidazol-1-yl)purine and 6-(2-dodecylimidazol-1-yl)purine. Glycosylation of 6-(2-hexylimidazol-1-yl)purine with the sugar chloride gave much lower stereoselectivity varying from an anomeric ratio (α/β) of 1:4.5-7.1 in a binary solvent mixture (CH₃CN/toluene, 1:1) to 1:0.6 in DMF or CH₃CN/DMF (1:1). An even weaker solvent effect was observed for coupling reactions between 6-(2-dodecylimidazol-1-yl)purine and the sugar chloride. The stereoselectivity ranged from an anomeric ratio (α/β) of 1:2.0 in the binary solvent mixture (CH₃CN/toluene, 1:1) to 1:0.6 in DMF and 1:0.39 in toluene. The coupling reaction was far from completion in toluene. Further attempts at coupling 6-(2-alkylimidazol-1-yl)purines with the sugar chloride in CH₂Cl₂ or a mixture of DCE/toluene (1:1) failed to give significant yields of reaction products.

In summary, the effects of the alkyl chains on stereoselectivity varied with the chain lengths. The sodium salt of 6-(2-propylimidazol-1-yl)purine was coupled with the sugar chloride in CH₃CN/toluene (1:1) to give a quantitative yield, with excellent stereoselectivity (de, 94%), and the β-anomer was exclusively formed in CH₃CN/CH₂Cl₂ (1:1, v/v). Increases in chain length resulted in decreases in both stereoselectivity and yield. The sodium salts of 6-(2-hexylimidazol-1-yl)purine and 6-(2-dodecylimidazol-1-yl)purine were coupled with the sugar chloride in CH₃CN/toluene (1:1) to give anomeric ratios (α/β) of 1:4.5-7.1 and 1:2.0, respectively.
Scheme 8. Stereoselectivity for glycosylation of the sodium salt of 21c

<table>
<thead>
<tr>
<th>Solvent 1/2</th>
<th>22/23</th>
<th>Yield%</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMF/DMF</td>
<td>1/1.4</td>
<td>78</td>
</tr>
<tr>
<td>CH$_3$CN/CH$_3$CN</td>
<td>4.2/1</td>
<td>50</td>
</tr>
<tr>
<td>CH$_3$CN/Toluene + CH$_3$CN</td>
<td>33.7/1</td>
<td>100</td>
</tr>
<tr>
<td>CH$_3$CN/CH$_2$Cl$_2$ + CH$_3$CN</td>
<td>1.0/0</td>
<td></td>
</tr>
<tr>
<td>Toluene/Toluene</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Scheme 9. Stereoselectivity for glycosylation of the sodium salt of 21a

<table>
<thead>
<tr>
<th>Solvent 1/2</th>
<th>24/25</th>
<th>Yield%</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMF/DMF</td>
<td>0.65/1</td>
<td></td>
</tr>
<tr>
<td>DMF/DMF + CH$_3$CN</td>
<td>0.63/1</td>
<td></td>
</tr>
<tr>
<td>CH$_3$CN/Toluene + CH$_3$CN</td>
<td>4.5-7.1/1</td>
<td></td>
</tr>
<tr>
<td>CH$_3$CN/CH$_2$Cl$_2$ + CH$_3$CN</td>
<td>3.4/1</td>
<td>43</td>
</tr>
</tbody>
</table>
Scheme 10. Stereoselectivity for glycosylation of the sodium salt of 21b

2.2.4.3. Effects of Solvent Mixtures: Polarity and Solubility

General trends in the solvent effects and the effects of alkyl chains are plotted in Figure 7. It is concluded that solvent mixtures for optimal coupling reactions should have dielectric constants around 20, and chain lengths of about 3 are optimal. These fulfill the requirements for a practical glycosylation of purines with high regio- and stereoselectivity, and also high yields.
Figure 7. Solvent effects (a) and effects of alkyl chains (b) on the stereoselectivity of glycosylation via the sodium salt method

Rates of S_N2 reactions depend on both the concentrations of reactants and the height of the free energy of activation barrier. Introduction of lipophilic groups increases the solubility of reactant species in less polar solvents. Solubilities of polar solutes are also increased in binary mixtures of polar and nonpolar solvents with resulting variations in the overall dielectric constants. The general trends of effects of alkyl groups on stereoselectivity might be mainly solubility phenomena, i.e., 6-(2-propylimidazol-1-yl)purine is dissolved best in solvents of suitable dielectric constant. The observed
solvent effects result from the differential solvation capabilities of solvents for the different reaction species.

Polar solvents can solvate polar species, and nonpolar species dissolve better in nonpolar solvents. Based on the ‘solvation rule’\textsuperscript{44,45} for $S_N$2 reactions, solvation may change the structure of an $S_N$2 transition state depending on the reaction type. “A change in solvent will not lead to a change in the structure of an $S_N$2 transition state (TS) if the charges on the two nucleophiles in the TS are the same, but will lead to a change in the structure of the TS when a negatively charged nucleophile and a neutral nucleophile are present in the TS”\textsuperscript{44} The effect is diminished when nucleophiles exist as ion pairs\textsuperscript{46,47}.

The glycosylation is of the first type. Because of the dispersal of charge during formation of the $S_N$2 transition state from the purine sodium salt and the sugar chloride, the lowering of its energy in polar solvents should be smaller than that of the charged nucleophiles. Thus, a decrease in the polarity of solvents usually decreases the free energy of activation of $S_N$2 reactions with charged nucleophiles that are less strongly solvated.

As mentioned before (Figure 1), glycosylation via an $S_N$1 pathway is diminished in nonpolar solvents. The rate determining step is formation of an oxocarbenium ion. The transition state resembles the cation according to Hammond’s postulate\textsuperscript{48} Thus, ionization of the neutral sugar chloride results in charge separation in the transition state. The transition state is more strongly solvated than the sugar chloride. Decreasing the polarity of solvents increases the $S_N$1 free energy of activation. Anomerization of the $\alpha$ sugar chloride (i.e., inversion of configuration) also occurs by an
$S_N1$ mechanism. Therefore, lowering the solvent polarity is an effective method to increase the formation of glycosylation product by an $S_N2$ pathway.

Thus, the observed trends of solvent effects for glycosylation are governed by at least two factors: solubility of the purine sodium salts and differential solvation. At one end, high polarity solvents favor the $S_N1$ pathway (anomerization of sugar chloride and $S_N1$ glycosylation) resulting in lower stereoselectivity; at the other end, the lower solubility of the salts leads to slower glycosylation, and the slow anomerization of the sugar chloride becomes significant.

Solvent mixtures gave extra benefits besides the variable dielectric constants, which are accessible with neat solvents. Preferential solvation in binary mixtures is well known.\textsuperscript{34-36} A polar solute interacts differently with the combined solvent molecules. The composition of solvents in the immediate vicinity of the solute ($X_{\text{local}}$) is different from the bulk composition ($X_{\text{bulk}}$). Wilson\textsuperscript{37} proposed a local composition (LC) to describe the microscopic structure, which has been extensively used to correlate vapor-liquid equilibrium data of binary and multi-component mixtures. Deng et al.\textsuperscript{38} correlated $^1\text{H}$ NMR chemical shifts of binary mixtures with compositions based on a local composition (LC) model. The derived energetic parameters reflect the general trends for the strengths of intermolecular interactions.

Prausnitz et al.\textsuperscript{39} proposed the following method for calculation of local composition in a binary mixture $(i, j)$.

With the definition of

$$\Lambda_{i,j} = \frac{V_i^L}{V_j^L} \exp\left[-(\lambda_{i,j} - \lambda_{i,i}) / RT \right]$$

(3)

the local volume fractions can be written as
where $\Lambda_{i,j}$ and $\Lambda_{i,i}$ are proportional to the i-j and i-i interaction energies.

The preferential solvation of reaction species results in alteration of the free energy of activation, which controls the ratio of two competitive reaction pathways. Kondo et al. measured the rates of the reaction of bromide ion and ethyl iodide in MeOH/CH$_3$CN and in MeOH/DMAc to evaluate the effects of preferential solvation of anions and of solvent-solvent interactions. The behavior of the rate constant was attributed largely to the specific solvation of bromide ion by methanol. It is obvious that small and highly charged nucleophiles are less solvated in dipolar aprotic solvents than in protic solvents due to the small volume of the nucleophile.

Preferential solvation in such a system also increases the solubility of polar solutes, which was very significant in supercritical binary mixtures. Therefore, a binary solvent mixture has the advantage of increased solvation ability, which is a partial solution for the problem of low solubility of purine sodium salts in less polar solvents.

Kawakami et al. reported the high stereoselectivity of coupling reactions, with modest yields, between the sodium salt of adenine and the chlorosugar in solvents of moderate polarity. Gerszberg et al. reported a synthesis of cladribine in neat acetone using the sodium salt method. By controlling reaction times, high stereoselectivities were attained by sacrificing yields. The low yields resulted from low solubility of the sodium salts of adenine and 6-amino-2-chloropurine. In the present work, the sodium salt was prepared in a polar solvent (CH$_3$CN, 1) to give well solvated ions. When the sugar chloride in the less polar solvent (toluene, 2) was added, the polar solvent was diluted. At
equilibrium, the solvent composition around the sodium salt ($X_{1,\text{local}}$) is larger than the bulk composition ($X_{1,\text{bulk}}$). Because the sugar chloride is much less polar than the sodium salt, the local environment around the salt is more polar than that around the sugar chloride due to preferential solvent aggregation.

The preferential solvation of sodium salts by polar solvent (1) increases the solubility of the salt. The decreased polarity in the environment around the sugar chloride benefits the stereoselectivity by minimizing anomerization and maximizing the percentage of the $S_N2$ pathway for glycosylation. Preferential solvation of the sodium salt should lower its energy, thus increasing the free energy of activation for $S_N2$ glycosylation; but this still should be much smaller than that in neat polar solvents, in harmony with the experimental results.

An abrupt change in stereoselectivity in the region of CH$_3$CN/DMF mixtures was observed, which suggested different types of solute-solvent interactions. Besides its higher polarity, DMF is a much stronger HBA ($\beta = 0.69$) than CH$_3$CN. CH$_3$CN is both a weak HBA ($\beta = 0.35$) and a weak HBD ($\alpha = 0.19$). The extra negative charge on the oxygen of DMF can further stabilize the transition state of $S_N1$ pathways via Lewis acid-base interactions, in harmony with the anomeric mixtures of obtained products. CH$_3$CN can solvate both oxocarbenium ions and sodium salts. Stabilization of the transition state of an $S_N1$ pathway by DMF should be much larger than stabilization by CH$_3$CN. Dilution of DMF with CH$_3$CN decreased the stabilization of the transition state for $S_N1$ pathways. However, with increased amounts of CH$_3$CN, stabilization of the sodium salts occurred with increased free energy of activation for the $S_N2$ pathway. CH$_3$CN can stabilize the transition state of $S_N1$ reactions, but to a lesser extent than DMF. Better stereoselectivity
was observed in mixtures of DMF/CH$_3$CN than in neat DMF for 6-(1,2,4-triazol-4-yl)purine, 6-(imidazol-1-yl)purine, and 6-(2-propylimidazol-1-yl)purine.

2.2.4.4. Experimental Evidence for Preferential Solvation of a Purine Sodium Salt in a Binary Solvent Mixture (CH$_3$CN/CH$_2$Cl$_2$)

(a) Variation of UV spectra with composition of solvents

(b) Space filling model for the solute molecule (MM2)
(c) UV of CH$_2$Cl$_2$ of different samples (Mallinckrodt Baker, Inc.)

(d) UV of solvents (Plotted using Aldrich data)

Figure 8. Preferential solvation of a purine sodium salt in CH$_3$CN/CH$_2$Cl$_2$
The preferential solvation of 2-chloro-6-(2-butylimidazol-1-yl)purine sodium salt in a binary solvent mixture (CH₃CN/CH₂Cl₂) was measured by UV spectra in dilute solution (~10⁻⁴ M). Significant deviations from linear behavior for both the absorbances at 228 and 296 nm were observed, and the shifts of λ_max also deviated from a linear relation. Both of these effects suggested a significant preferential aggregation of solvents around the solute (Figures 8, 9).

This sodium salt acts as an amphiphile. The strength of interactions in solution decreases approximately in the following order: solute sodium salt region/CH₃CN > CH₃CN/CH₃CN > solute lipophilic region/CH₃CN ≥ CH₃CN/CH₂Cl₂ ≥ solute lipophilic region/CH₂Cl₂ > CH₂Cl₂/CH₂Cl₂. Equilibria are reached when the enthalpy changes due to these favorable interactions are balanced by the loss of entropy caused by local solvent aggregation around the solute. The preferential solvation caused by competitive interactions among these components increased X_{local} (CH₃CN) in the vicinity of the sodium salt region, which is supported by the observed significant deviation from linear relations for absorbance at 296 nm. CH₂Cl₂ was concentrated around the solute lipophilic region, and this decreased the X_{bulk} (CH₂Cl₂), which agreed with the negative absorbance observed in the CH₃CN/CH₂Cl₂ (2:1) solution [Fig. 8(a)].
Figure 9. Solvatochromism of the sodium salt of 2-chloro-6-(2-butylimdazol-1-yl)purine

2.3. Attempted Glycosylation of Glucopyranose Derivatives via the Sodium Salts of 6-(Imidazol-1-yl)purines

Attempts to couple 6-(imidazol-1-yl)purine with 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride gave low yields of nucleosides due to incomplete reactions, probably resulting from the crude sugar chloride. However, only the 9-β-nucleoside was
obtained. 2,3,4,6-Tetra-O-benzoyl-α-D-glucopyranosyl bromide can be easily prepared\textsuperscript{1} and purified by the procedure analogous to a reported method for L-glucopyranose.\textsuperscript{51} Glycosylation of this sugar bromide using the sodium salt method was then tried.

Acylation of D-glucose with PhCOCl in pyridine gave perbenzoylated glucose (88%), which was treated with HBr/HOAc (30% w/w) in CH\textsubscript{2}Cl\textsubscript{2} to give the sugar bromide in high yield (90%).

Coupling of the sodium salts of 6-(imidazol-1-yl)purines with the sugar bromide gave low yields of nucleosides (30%), and the reactions were incomplete. Glycosylation of 6-(2-hexylimidazol-1-yl)purine or 2-chloro-6-(2-propylimidazol-1-yl)purine gave similar yields (30%). Glycosylation of 2-amino-6-(imidazol-1-yl)purine or 2-acetamido-6-(imidazol-1-yl)purine gave even poorer yields, 11% and 16%, respectively. Elevation of the reaction temperature caused decomposition of the reactants. Reactions using K\textsubscript{2}CO\textsubscript{3} as the base gave even lower yields (Scheme 11).

![Scheme 11](image)

**Scheme 11. Glycosylation of a glucopyranosyl bromide via the sodium salts of 6-(imidazol-1-yl)purines**
3. Conclusions

Glycosylation of a 2-deoxy furanose derivative using the sodium salt method usually gave a mixture of both anomers. Introduction of lipophilic groups to increase the solubility of the purine sodium salts in less polar solvents and differential solvation effects in binary solvent mixtures were utilized to improve the stereoselectivity of glycosylation. With suitable alkyl chains, regiospecific and highly stereoselective glycosylations with 2-deoxy-3,5-di-O-(p-toluoyl)-α-D-erythro-pentofuranosyl chloride were achieved.

As observed in Chapter 2, glycosylation with a halosugar by the sodium salt method is much faster for 2-deoxyfuranosides than for pyranosides. The less successful glycosylation with a ribofuranosyl chloride might be due to impurities in the sugar chloride mixture.

4. Experimental Section

9-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)-6-(imidazol-1-yl)purine (3a)

6-(Imidazol-1-yl)purine (52 mg, 0.28 mmol) was suspended in a solution of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (160 mg, 0.32 mmol) in dried CH$_3$CN (10 mL). Stannic chloride (0.10 mL, 0.22 g, 0.85 mmol) was added, and the mixture very rapidly became a clear solution. The solution was stirred at ambient temperature for 4 h. NaHCO$_3$ (0.8 g) and H$_2$O (0.1 mL) were added sequentially, and the suspension was stirred for 1 h. The clear solution layer was separated, and the residue was extracted with CH$_3$CN. The extracts and the solution layer were combined, and volatiles were evaporated in vacuo. The residue was chromatographed (CH$_2$Cl$_2$/MeOH, 1:90 → 1:15) to give 3a (179 mg, quantitative): $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 4.72 (dd, $J = 4.3, 12.2$ Hz,
1H), 4.88 (br s, 1H), 4.95 (dd, J = 3.0, 12.3 Hz, 1H), 6.29 (“t”, J = 5.2 Hz, 1H), 6.47–6.50 (m, 2H), 7.24 (s, 1H), 7.35–7.60 (m, 9H), 7.93 (d, J = 7.6 Hz, 2H), 8.03 (d, J = 7.6 Hz, 2H), 8.07 (d, J = 7.6 Hz, 2H), 8.28 (s, 1H), 8.35 (s, 1H), 8.65 (s, 1H), 9.13 (s, 1H); 13C NMR (125 MHz, CDCl₃) δ 165.0, 164.3, 164.1, 152.1, 151.5, 144.9, 142.2, 136.6, 132.9, 132.8, 132.4, 129.7, 128.8, 128.7, 128.2, 127.5, 127.2, 122.0, 116.3, 86.4, 79.9, 72.9, 70.3, 62.3; HRMS m/z 653.1749 (MNa⁺ [C₃₄H₂₆N₆O₇Na] = 653.1761).

2-Acetamido-9-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-6-(imidazol-1-yl)purine (3c)

2-Acetamido-6-(imidazol-1-yl)purine (62 mg, 0.26 mmol) was suspended in a solution of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (160 mg, 0.32 mmol) in dried CH₃CN (10 mL). SnCl₄ (0.10 mL, 0.22 g, 0.85 mmol) was added, and the mixture quickly became clear. The solution was stirred at ambient temperature overnight. NaHCO₃ (0.8 g) and H₂O (0.1 mL) were added sequentially, and the suspension was stirred for 1 h. The clear solution layer was separated, and the residue was extracted with CH₃CN. The extracts and the solution layer were combined, and volatiles were evaporated in vacuo. The residue was chromatographed (EtOAc/hexanes, 7:3 → 85:15) to give 3c (24 mg, 13%): ¹H NMR (500 MHz, CDCl₃) δ 2.51 (s, 3H), 4.76 (dd, J = 4.9, 12.2 Hz, 1H), 4.91 (br s, 1H), 4.97 (dd, J = 3.4, 12.2 Hz, 1H), 6.34 (s, 1H), 6.37–6.39 (m, 2H), 7.24 (s, 1H), 7.36–7.61 (m, 9H), 7.93 (d, J = 8.0 Hz, 2H), 7.99 (d, J = 7.6 Hz, 2H), 8.02 (d, J = 8.0 Hz, 2H), 8.10 (s, 1H), 8.27 (s, 1H), 8.37 (s, 1H), 9.05 (s, 1H); ¹³C NMR (125 MHz, CDCl₃) δ 165.1, 164.3, 164.2, 152.9, 151.4, 145.1, 141.4, 136.6, 132.9, 132.8, 132.4, 129.9, 128.8, 128.6, 128.1, 127.58, 127.5, 127.3, 118.5, 116.4, 86.8, 79.6, 73.2, 70.4, 62.3, 24.3; HRMS m/z 710.1971 (MNa⁺ [C₃₆H₂₉N₇O₈Na] = 710.1975).
Further elution gave byproduct 4c (66 mg, 35%): HRMS m/z 728.2090 (MNa+ [C₃₆H₃₁N₇O₉Na] = 728.2081).

The reaction was repeated at 65 °C for 2 h. The same workup and purification procedure gave 3c (24 mg, 13%) only.

9-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)-6-(2,3-dihydro-2-hydroxyimidazol-1-yl)purine (4a)

BSA (0.26 mL, 0.21 g, 1 mmol) was added to a suspension of 6-(imidazol-1-yl)purine (1a) (93 mg, 0.5 mmol) in dried DCE (10 mL), and the mixture was stirred at 80 °C. A clear solution resulted in ~2 h. Volatiles were evaporated in vacuo, and the residue was dissolved in dried toluene (5 mL). 1-0-Acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (311 mg, 0.6 mmol) dissolved in toluene (5 mL) and TMSOTf (0.126 mL, 0.137 g, 0.81 mmol) were added, and the solution was stirred for 2 h. The solution was cooled, volatiles were evaporated in vacuo, and the residue was stirred with NaHCO₃/H₂O/CH₂Cl₂ (130 mL/60 mL) overnight. The organic phase was dried (Na₂SO₄) and evaporated to dryness. The residue was chromatographed (25g silica gel, MeOH/CH₂Cl₂, 1:30 → 1:12) to give 4a (0.265 g, 84%) as a mixture of two diastereomers: 1H NMR (500 MHz, CDCl₃) δ 4.52 (4.58) (dd, J = 3.0, 12.2 Hz, 1H, H5’), 4.70 (br s, 1H, H4’), 4.83–4.86 (m, 1H, H5”), 5.48 (5.62) (d, J = 5.5/6.4 Hz, 1H), 5.80–5.92 [m, 2H (H2’,H3’)/3H (H1’, H2’, H3’)], 6.62 (d, J = 7.4 Hz, 1H, H1’), 7.30–7.43 (m, 6H, Ph), 7.54–7.60 (m, 3H, Ph), 7.77–8.00 [m, 7H (6H₇ₘ and 1Hₐₗkene)/8H (6Hₚₘ and 2Hₐₗkene)], 8.28 (d, J = 7.7 Hz, 1Hₐₗkene), 8.31 (s, 1H), 8.52 (s, 1H); HRMS m/z 671.1882 (MNa+ [C₃₄H₂₈N₆O₈Na] = 671.1866.

178
The reaction was repeated with 1a (0.186 g, 1.0 mmol). Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH₂Cl₂, 1:30) to give the protonated imidazonium salt (3aH⁺) (778 mg): ¹H NMR (300 MHz, CDCl₃) δ 10.46 (s, 1H), 8.78 (s, 1H), 8.64 (s, 1H), 8.23 (s, 1H), 8.12 (d, J = 1.5 Hz, 1H), 7.89–8.03 (m, 6H), 7.22–7.61 (m, 9H), 6.81 (d, J = 3.6 Hz, 1H), 6.07–6.10 (m, 1H), 5.99 (t, J = 5.4 Hz, 1H), 5.85 (br s, 1H), 5.00–5.09 (m, 2H), 4.76–4.80 (m, 1H); HRMS m/z 653.1742 (MNa⁺ [C₃₄H₂₆N₆O₇Na] = 653.1761). The salt was stirred with NaHCO₃/H₂O//CH₂Cl₂ to give 4a (511 mg, 81%).

2-Amino-9-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-6-(2,3-dihydro-2-hydroxyimidazol-1-yl)purine (4b)

BSA (0.26 mL, 0.21 g, 1.0 mmol) was added to a suspension of 2-amino-6-(imidazol-1-yl)purine (0.1 g, 0.5 mmol) in dried DCE (10 mL), and the mixture was stirred at 80 °C for ~1.5 h. Volatiles were evaporated in vacuo, and the residue was dissolved in 5 mL of dried toluene. 1-O-Acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (0.31 g, 0.60 mmol) in toluene (5 mL) and TMSOTf (0.15 mL, 0.164 g, 0.97 mmol) were added, and the mixture was stirred for 4 h. The solution was cooled, and volatiles were evaporated in vacuo. The residue was stirred with NaHCO₃/H₂O//CH₂Cl₂ (130 mL/60 mL) overnight, and the organic phase was dried (Na₂SO₄) and evaporated to dryness. The residue was chromatographed (25g silica gel, MeOH/CH₂Cl₂, 1:30 → 1:15) to give 4b as a mixture of diastereomers (0.237g, 72%): ¹H NMR (500 MHz, CDCl₃) δ 4.55–4.59 (m, 1H, H5’), 4.66–4.68 (m, 3H, H4’, NH₂), 4.79–4.82 (m, 1H, H5”), 5.38 (5.53) (d, J = 5.8/6.7 Hz, 1H), 5.77–5.90 [m, 2H (H2’,H3’)/3H (H1’, H2’, H3’)], 6.59 (d, J = 7.6 Hz, 1H, H1’), 7.32–7.44 (m, 6H, Ph), 7.53–7.58 (m, 3H, Ph), 7.83–8.00 [m, 8H (6Hₚ and
2H\textsubscript{alkene}], 8.28 (s, 1H), 8.49 (s, 1H); HRMS \textit{m/z} 686.1976 (MNa\textsuperscript{+} [C\textsubscript{34}H\textsubscript{29}N\textsubscript{7}O\textsubscript{8}Na] = 686.1969).

3-(2,3,5-Tri-\textit{O}-benzoyl-\textit{β}-D-ribofuranosyl)-1-(9-isopropylpurin-6-yl)imidazolium Triflate (3d)

BSA (0.14 mL, 0.21 g, 0.50 mmol) was added to a suspension of 6-(imidazol-1-yl)-9-isopropylpurine (58 mg, 0.26 mmol) in dried DCE (10 mL), and the mixture was stirred at 80 °C for 1 h. Volatiles were evaporated in vacuo, and the residue was dissolved in dried toluene (5 mL). 1-\textit{O}-Acetyl-2,3,5-tri-\textit{O}-benzoyl-\textit{β}-D-ribofuranose (0.152 g, 0.3 mmol) in toluene (5 mL) and TMSOTf (0.1 mL, 0.109 g, 0.65 mmol) were added. The mixture was stirred for 4 h (TLC showed the product as the major spot below a minor spot). The solution was cooled, and volatiles were removed in vacuo. The residue was chromatographed (25 g silica gel, MeOH/CH\textsubscript{2}Cl\textsubscript{2}, 1:30) to give 3d: \textit{\textit{1}H NMR (500 MHz, CDCl}\textsubscript{3}) \textit{δ} 1.67 (s, 3H), 1.68 (s, 3H), 4.75 (dd, \textit{J} = 2.8, 12.0 Hz, 1H), 4.95–5.03 (m, 3H), 5.98–6.02 (m, 2H), 7.04 (d, \textit{J} = 4.3 Hz, 1H), 7.33–7.61 (m, 9H), 7.99–8.10 (m, 7H), 8.21 (s, 1H), 8.75 (s, 1H), 8.81 (s, 1H), 10.71 (s, 1H); HRMS \textit{m/z} 673.2427 (M\textsuperscript{+} [C\textsubscript{37}H\textsubscript{33}N\textsubscript{6}O\textsubscript{7} = 673.2411]).

2-Amino-9-(2,3,5-tri-\textit{O}-benzoyl-\textit{β}-D-ribofuranosyl)-6-(imidazol-1-yl)purine (6)

To a solution of 1-\textit{O}-acetyl-2,3,5-tri-\textit{O}-benzoyl-\textit{β}-D-ribofuranose (2.04 g, 4.1 mmol) and HOAc (0.82 mL, 0.86 g, 14.3 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (10 mL) was added SOCl\textsubscript{2} (2.1 mL, 3.43 g, 28.8 mmol) at 0 °C. The solution was allowed to warm to ambient temperature and stirred for 18 h. Additional SOCl\textsubscript{2} (1.0 mL, 1.63 g, 13.7 mmol) and HOAc (0.4 mL, 0.42 g, 3.53 mmol) were added, and stirring was continued overnight.
(reaction incomplete, TLC). Volatiles were evaporated, and toluene was added and then evaporated in vacuo. The residue was dried under vacuum to give a solid (1.88 g) containing 1-O-acetyl-2,3,5-tri-O-benzoyl-α/β-D-ribofuranose and 2,3,5-tri-O-benzoyl-α/β-D-ribofuranosyl chloride (~1:6). The solid was dissolved in DMF (20 mL), and used without further purification.

A mixture of 2-amino-6-(imidazol-1-yl)purine (0.1 g, 0.5 mmol) and sodium hydride (26 mg, 60% w/w suspension, 0.6 mmol) in dried DMF (5 mL) was stirred at ambient temperature under N₂ for 4 h. The resulting sodium salt was treated with the crude sugar chloride (~0.2 M, 3.8 mL) in DMF, and the mixture was stirred for several days (reaction incomplete, TLC). Volatiles were evaporated in vacuo, and the residue was chromatographed (25 g silica gel, CH₂Cl₂ → MeOH/CH₂Cl₂, 1:90 → 1:60) to give 6 (57 mg, 18%): HRMS m/z 668.1870 (MNa⁺ [C₃₄H₂₇N₇O₇Na] = 668.1870).

**General Method 1 for Preparation of 9-[2-deoxy-3,5-di-O-(p-toluoyl)-D-erythro-pentofuranosyl]-6-heteroarylpurines (12-15, 22-27)**

A mixture of the 6-heteroarylpurine (1 mmol) and sodium hydride (0.06 g, 60% w/w suspension, 1.5 mmol) in the dried polar solvent A was stirred at ambient temperature under positive nitrogen pressure for 2 h. A solution of 10 (1.8 mmol) in the dried less-polar solvent B was added with a syringe, and the mixture was then stirred for 22 h. Volatiles were evaporated in vacuo.
General Method 2 for Preparation of 9-[2-deoxy-3, 5-di-O-(p-toluoyl)-D-erythro-pentofuranosyl]-6-heteroarylpurines (22, 24-27)

A mixture of the 6-(2-alkylimidazol-1-yl)purine (1mmol) and sodium hydride (60% w/w suspension, 1.5 mmol) in dried CH$_3$CN (10 mL) was stirred at ambient temperature under N$_2$ for 8 h. The solution was chilled to 0 °C, and a solution of 10 (1.8 mmol) in cold, dried CH$_2$Cl$_2$ (10 mL, 0 °C) was added with a syringe. The reaction mixture was stirred for 22 h and allowed to gradually warm to ambient temperature. Volatiles were evaporated in vacuo, and the residue was chromatographed (25g silica gel, MeOH/CH$_2$Cl$_2$, 1:30).

9-[2-Deoxy-3,5-di-O-(p-toluoyl)-α/β-D-erythro-pentofuranosyl]-6-(1,2,4-triazol-4-yl)purines (12,13)

<table>
<thead>
<tr>
<th>A</th>
<th>#</th>
<th>A + B</th>
<th>α/β</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMF (5 mL)</td>
<td>1</td>
<td>DMF (15 mL)</td>
<td>1.1:1.0</td>
<td>58</td>
</tr>
<tr>
<td>DMF (5 mL)</td>
<td></td>
<td>DMF (5 mL)/CH$_3$CN (25 mL)</td>
<td>1.0:2.5</td>
<td>54</td>
</tr>
<tr>
<td>DMF (5 mL)</td>
<td></td>
<td>CH$_3$CN (35 mL)</td>
<td>1.6:1.0</td>
<td>40</td>
</tr>
<tr>
<td>DMF (5 mL)</td>
<td>2</td>
<td>DMF (10 mL), 0 – 20 °C</td>
<td>1.0:1.3</td>
<td>63</td>
</tr>
<tr>
<td>DMF (5 mL)</td>
<td>3</td>
<td>DMF (8 mL)/CH$_3$CN (15 mL)</td>
<td>1.0:2.9</td>
<td>83</td>
</tr>
<tr>
<td>DMF (5 mL)</td>
<td>4</td>
<td>DMF (10 mL)</td>
<td>1.0:1.8</td>
<td></td>
</tr>
<tr>
<td>DMF (5 mL)</td>
<td>5</td>
<td>DMF (10 mL)</td>
<td>1.0/1.0</td>
<td>53</td>
</tr>
</tbody>
</table>

Table 3. Stereoselectivity for glycosylation of the sodium salt of 6-(1,2,4-triazol-4-yl)purine
The sodium salt of 6-(1,2,4-triazol-4-yl)purine (0.19 g, 1 mmol) in solvent A was treated with 10 (0.58 g, 1.5 mmol) in solvent B by general method 1. The derived residue was chromatographed to give the anomeric mixture of 12 and 13: $^1$H NMR (300 MHz, CDCl$_3$) δ 6.75 (dd, $J = 1.9$, 6.3 Hz, 1H, H$_{1',\alpha}$), 6.64 (dd, $J = 5.9$, 7.6 Hz, 1H, H$_{1',\beta}$).

9-[2-Deoxy-3,5-di-O-($p$-toluoyl)-$\alpha/\beta$-D-erythro-pentofuranosyl]-6-(imidazol-1-yl)purines (14,15)

The sodium salt of 6-(imidazol-1-yl)purine (0.19 g, 1 mmol) in solvent A was treated with 10 (0.58 g, 1.5 mmol) in solvent B by general method 1. The derived residue was chromatographed to give the anomeric mixture of 14 and 15: $^1$H NMR (300 MHz, CDCl$_3$) δ 6.74 (d, $J = 5.9$ Hz, 1H, H$_{1',\alpha}$), 6.65 (t, $J = 6.7$ Hz, 1H, H$_{1',\beta}$).

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>#</td>
<td>A + B</td>
<td>α/β*</td>
</tr>
<tr>
<td>DMF (5 mL)</td>
<td>1</td>
<td>DMF (10 mL)</td>
<td>1.6:1.0</td>
</tr>
<tr>
<td>DMF (5 mL)</td>
<td>2</td>
<td>DMF (5 mL)/CH$_3$CN (5 mL)</td>
<td>1.0:1.0</td>
</tr>
<tr>
<td>CH$_3$CN (5 mL)</td>
<td>3</td>
<td>CH$_3$CN (10 mL)</td>
<td>1.7:1.0</td>
</tr>
<tr>
<td>CH$_3$CN (5 mL)</td>
<td>4</td>
<td>CH$_3$CN (5 mL)/Toluene (5 mL)</td>
<td>1.0:6.9 (1.0:5.0)</td>
</tr>
</tbody>
</table>

*Chromatography (MeOH/CH$_2$Cl$_2$, 1:20); Values in parentheses are before column chromatography.

Table 4. Stereoselectivity for glycosylation of the sodium salt of 6-(imidazol-1-yl)purine

2-Hexylimidazole (18a)

The procedure to prepare 18a from 17 was analogous to that reported by Katritzky and his coworkers. 33 A sample of 17 (6.28 g, 50 mmol) was dissolved in dried
THF (200 mL), and the solution was cooled to -78 °C under N₂. Butyllithium/hexanes (1.6 M, 40 mL) was added dropwise, and the resulting solution was stirred at -78 °C for 1.5 h. 1-Iodohexane (8 mL, 11.5 g, 54 mmol) in THF (25 mL) was added at -78 °C, and the reaction mixture was stirred for 30 h and allowed to warm to ambient temperature.

HCl/H₂O (37% w/w, 30 mL) was added, and the solution was stirred overnight. Another 25 ml of HCl/H₂O (37% w/w) was added, and the solution was stirred for 2 days. The reaction mixture was neutralized with NaHCO₃, and volatiles were concentrated in vacuo. The suspension was extracted with CHCl₃ (3 x 100 mL), and the combined organic phase was dried (Na₂SO₄). Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH₂Cl₂, 1:20) to give 18a (6.48 g, 85%): 

1H NMR (300 MHz, CDCl₃) δ 7.10 (s, 2H), 3.00 (t, J = 7.7 Hz, 2H), 1.81 (quint, J = 7.4 Hz, 2 H), 1.45–1.27 (m, 6H), 0.86 (t, J = 6.8 Hz, 3H); 13C NMR (75 MHz, CDCl₃) δ 148.6, 119.1, 31.5, 29.0, 28.7, 27.1, 22.7, 14.2; HRMS m/z 152.1307 (M⁺ [C₉H₁₆N₂] = 152.1313).

2-Dodecylimidazole (18b)

The procedure to prepare 18a from 17 was analogous to that reported by Katrizky and his coworkers. A sample of 17 (1.32 g, 10.6 mmol) was dissolved in dried THF (40 mL), and the solution was cooled to -78 °C under N₂. Butyllithium (1.6 M, 8.0 mL) was added dropwise, and the resulting solution was stirred at -78 °C for 1.5 h. 1-Iodododecane (4.0 mL, 4.8 g, 16.2 mmol) in THF (5 mL) was added at -78 °C, and the reaction mixture was stirred for 30 h and allowed to warm to ambient temperature. HCl/H₂O (37% w/w, 2 x 6 mL) was added, and the solution was stirred overnight and neutralized with NaHCO₃. Volatiles were evaporated in vacuo, and the residue was extracted with CHCl₃ (3 x 100 mL). The combined organic phase was dried (Na₂SO₄),
and volatiles were evaporated in vacuo. The residue was chromatographed (EtOAc) to give a solid (1.96 g, 79%), which was recrystallized (CH$_2$Cl$_2$) to give purified 18b (1.51 g, 59%): mp 76.5-77.5 °C; $^1$H NMR (300 MHz, CDCl$_3$) δ 9.48 (s, 1H), 6.97 (s, 2H), 2.76 (t, $J = 7.5$ Hz, 2H), 1.75 (quint, $J = 7.5$ Hz, 2H), 1.33–1.27 (m, 18H), 0.90 (t, $J = 6.8$ Hz, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 149.18, 121.85, 32.18, 29.94, 29.90, 29.82, 29.63, 28.92, 28.90, 22.95, 14.39; HRMS m/z 236.2240 (M$^+$ [C$_{15}$H$_{28}$N$_2$] = 236.2252). Anal. Calcd for C$_{15}$H$_{28}$N$_2$: C, 76.21; H, 11.94; N, 11.85. Found: C, 76.42; H, 12.00; N, 12.02.

Early chromatographic fractions contained 1,2-bis(dodecyl)imidazole: $^{13}$C NMR (75 MHz, CDCl$_3$) δ 147.4, 120.7, 119.2, 48.0, 32.2, 30.5, 29.9, 29.8, 29.73, 29.70, 29.60, 29.40, 29.36, 29.28, 29.27, 28.3, 26.7, 25.2, 22.9, 14.4; LRMS m/z 404 (M$^+$ [C$_{27}$H$_{52}$N$_2$] = 404).

6-(2-Hexylimidazol-1-yl)purine (21a)

2',3',5'-Tri-O-acetylinosine (3.93 g, 10 mmol) was added to a stirred suspension of crude 18a (6.48 g, 42.7 mmol), Ph$_3$P (6.41 g, 21 mmol), I$_2$ (5.36 g, 20.7 mmol), and EtN(i-Pr)$_2$ (9.9 mL, 7.36 g, 56.7 mmol) in dried toluene (100 mL), and the mixture was stirred at 95 °C for 12 h. Volatiles were evaporated in vacuo, and the residue was extracted with boiling EtOAc. The combined EtOAc extracts were evaporated to dryness, and the residue was chromatographed (MeOH/CH$_2$Cl$_2$, 1:40). The derived solid was dissolved in AcOH (387 mL). AcCl (4.2 mL, 4.64 g, 59 mmol) was added, and the solution was stirred at 65 °C for 2 days. Volatiles were evaporated in vacuo, and the residue was dissolved in CHCl$_3$ and washed (NaHCO$_3$/H$_2$O). The combined organic layers were dried (Na$_2$SO$_4$). Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH$_2$Cl$_2$, 1:20). Recrystallization (MeOH) gave 21a (1.1 g,
41%): mp 224.5-225 °C; UV (MeOH) max 278 nm (ε 12 200), min 232 nm (ε 2200); ¹H NMR (500 MHz, DMSO- d₆) δ 13.89 (s, 1H), 8.84 (s, 1H), 8.68 (s,1H), 8.33 (s,1 H), 7.04 (d, J = 1.5 Hz,1H), 3.18 (t, J = 7.4 Hz, 2H), 1.66 (quint, J = 7.6 Hz, 2H), 1.32 (quint, J = 7.1 Hz, 2H), 1.20–1.24 (m, 4H), 0.82 (t, J = 7.1 Hz, 3H); ¹³C NMR ( 125 MHz, DMSO-d₆) δ 154.8, 151.2, 149.3, 146.1, 145.0, 127.8, 122.8, 120.6, 30.9, 29.4, 28.3, 27.3, 22.0, 13.8; HRMS (EI) m/z 270.1580 (M⁺ [C₁₄H₁₈N₆] = 270.1593). Anal. Calcd for C₁₄H₁₈N₆: C, 62.20; H, 6.71; N, 31.09. Found: C, 62.32; H, 6.86; N, 31.25.

6-(2-Dodecylimidazol-1-yl)purine (21b)

2',3',5'-Tri-O-acetylinosine (0.1g, 0.25 mmol) was added to a stirred suspension of 18b (0.21 g, 0.9 mmol), Ph₃P (0.16 g, 0.6 mmol), I₂ (0.14 g, 0.52 mmol), and EtN(i-Pr)₂ (0.22 mL, 0.16 g, 1.26 mmol), and stirring was continued at 95 °C for 12 h. Volatiles were evaporated in vacuo, and the residue was extracted with boiling EtOAc. The combined EtOAc extracts were evaporated to dryness, and the residue was chromatographed (EtOAc/hexanes, 1:1→ EtOAc). The derived solid was dissolved in AcOH (10 mL), and AcCl (0.11 mL, 103 mg, 1.5 mmol) was added. The solution was stirred at 65 °C overnight, and evaporated to dryness. The residue was dissolved in CHCl₃ and washed with saturated NaHCO₃/H₂O (3 x 50 mL). The combined organic layers were dried (Na₂SO₄), and volatiles were evaporated in vacuo. The residue was chromatographed (MeOH/CH₂Cl₂, 1:12) to give a solid containing 2-dodecylimidazole. Recrystallization (MeOH) gave 21b (48 mg, 54%): mp 210-210.5 °C; UV (MeOH) max 278 nm (ε 14 000), min 242 nm (ε 6000); ¹H NMR (300 MHz, DMSO- d₆) δ 13.87 (s, 1H), 8.83 (s, 1H), 8.67 (s, 1H), 8.33 (s, 1H), 7.04 (s, 1H), 3.18 (t, J = 7.3 Hz, 2H), 1.66 (quint, J = 7.3 Hz, 2H), 1.29–1.20 (m, 18H), 0.84 (t, J = 7.0 Hz, 3H); ¹³C NMR (125
MHz, CDCl$_3$) $\delta$ 154.90, 151.22, 149.24, 146.14, 145.04, 127.79, 122.85, 120.60, 31.26, 29.34, 28.96, 28.86, 28.66, 27.35, 22.06, 13.92; HRMS $m/z$ 354.2525 ($M^+$ $[C_{20}H_{30}N_6] = 354.2532$). Anal. Calcd for C$_{20}$H$_{30}$N$_6$: C, 67.76; H, 8.53; N, 23.71. Found: C, 67.90; H, 8.66; N, 23.86.

**6-(2-Propylimidazol-1-yl)purine (21c)**

A suspension of 2’,3’,5’-tri-O-acetylinosine (1.58 g, 4.0 mmol), 2-propylimidazole (1.60 g, 14.4 mmol), Ph$_3$P (2.58 g, 9.6 mmol), I$_2$ (2.14 g, 8.32 mmol), and EtN(-Pr)$_2$ (3.6 mL, 2.67 g, 20.2 mmol) in dried toluene (40 mL) was stirred at 95 °C for 4 h. Volatiles were evaporated in vacuo, and the residue was extracted with boiling EtOAc. The combined extracts were evaporated to dryness, and the residue was chromatographed (CH$_2$Cl$_2$/MeOH, 1:40) to give a solid contaminated with Ph$_3$PO. This material was dissolved in AcOH (160 mL), and AcCl (2.2 mL, 2.43 g, 31 mmol) was added. The solution was stirred at 65 °C overnight, and volatiles were evaporated in vacuo. The residue was dissolved in CH$_2$Cl$_2$ and extracted with 0.1 N NaOH/H$_2$O. The aqueous layer was washed (CH$_2$Cl$_2$), and precipitation with CO$_2$ followed by filtration and thorough washing (H$_2$O) gave a solid (0.66 g, 72%). This material was dissolved in MeOH and decolorized with charcoal. Recrystallization (MeOH) gave **21c** as a colorless solid: mp 242.5-243.5 °C; UV (MeOH) max 278 nm ($\varepsilon$ 13 700), min 235 nm ($\varepsilon$ 5000); $^1$H NMR (300 MHz, DMSO-d$_6$) $\delta$ 13.90 (br s, 1H), 8.86 (s, 1H), 8.69 (s, 1H), 8.36 (s, 1H), 7.07 (d, $J = 1.5$ Hz, 1H), 3.18 (t, $J = 7.3$ Hz, 2H), 1.72 (sext, $J = 7.3$ Hz, 2H), 0.93 (t, $J = 7.3$ Hz, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 155.5, 152.0, 149.8, 146.9, 145.8, 128.5, 123.6, 121.4, 32.2, 21.5, 14.5; HRMS $m/z$ 228.1109 ($M^+$ $[C_{11}H_{12}N_6] = 228.1123$). Anal. Calcd for C$_{11}$H$_{12}$N$_6$: C, 57.88; H, 5.30; N, 36.82. Found: C, 58.09; H, 5.19; N, 37.00.
9-[2-Deoxy-3,5-di-O-(p-toluoyl)-β-D-erythro-pentofuranosyl]-6-(2-propylimidazol-1-yl)purine (22)

The sodium salt of 21c (55 mg, 0.24 mmol) in dried CH₃CN (5 mL) was treated with 10 (0.15 g, 0.39 mmol) in toluene (5 mL) by general method 1. The residue was chromatographed (25 g silica gel, MeOH/CH₂Cl₂, 1:12) to give 22 [quantitative, containing traces of α-anomer (α/β ~1:34)]. Recrystallization (EtOAc) gave 22 (68.7 mg, 53%): mp 197-197.5 °C.; UV(MeOH) max 242, 276 nm (ε 31 200, 12 500), min 223, 263 nm (ε 16 400, 9700); ¹H NMR (500 MHz, CDCl₃) δ 8.80 (s, 1H), 8.41 (s, 1H), 8.28 (s, 1H), 8.00 (d, J = 8.3 Hz, 2H), 7.89 (d, J = 8.3 Hz, 2H), 7.31 (d, J = 8.3 Hz, 2H), 7.21 (d, J = 8.3 Hz, 2H), 7.12 (s, 1H) 6.65 (dd, J = 6.2, 8.1 Hz, 1H), 5.85–5.87 (m, 1H), 4.68–4.83 (m, 3H), 3.30 (t, J = 7.3 Hz, 2H), 3.17–3.23 (m, 1H), 2.91–2.95 (m, 1H), 2.47 (s, 3H), 2.39 (s, 3H), 1.85 (sext, J = 7.5 Hz, 2H), 1.03 (t, J = 7.3 Hz, 3H); NOE difference: irradiation at H1’ gave enhancement of the H4’ (small), H8 and H2’,2’’ signals; ¹³C NMR (125 MHz, CDCl₃) δ 166.4, 166.2, 153.1, 152.1, 151.0, 148.1, 144.9, 144.6, 142.4, 130.1, 129.8, 129.6, 129.5, 128.8, 126.8, 126.5, 124.6, 120.8, 85.5, 83.6, 75.2, 64.1, 38.3, 32.8, 22.0, 21.9, 21.5, 14.3; HRMS m/z 603.2347 (MNa⁺ [C₃₂H₃₂N₆O₅Na] = 603.2332); Anal. Calcd for C₃₂H₃₂N₆O₅: C, 66.20; H, 5.56; N, 14.47. Found: C, 66.59; H, 5.67; N, 14.62.

The reaction was repeated with general method 2. The sodium salt of 21c (66 mg, 0.25 mmol) in dried CH₃CN (10 mL) was treated with 10 (0.29 g, 0.75 mmol) in CH₂Cl₂ (10 mL) by general method 2 for 6 h (reaction complete, TLC). Sampling of the reaction mixture showed no α-nucleoside by ¹H NMR (500 MHz). Volatiles were evaporated. The residue was chromatographed (EtOAc/hexanes ~1:1→7:3) to give 22 (96 mg, 57%).
<table>
<thead>
<tr>
<th>#</th>
<th>A</th>
<th>A + B</th>
<th>α/β</th>
<th>%*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DMF (5 mL)</td>
<td>DMF (10 mL)</td>
<td>1.4:1</td>
<td>78</td>
</tr>
<tr>
<td>2</td>
<td>DMF (5 mL)</td>
<td>DMF (5 mL)/CH₃CN (25 mL)</td>
<td>1:1.2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>CH₃CN (10 mL)</td>
<td>CH₃CN (25 mL)</td>
<td>1.0:4.2</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>toluene (20 mL)</td>
<td>toluene (30 mL)</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>CH₃CN (10 mL)</td>
<td>CH₃CN (10 mL)/toluene (10 mL)</td>
<td>1:20</td>
<td>57</td>
</tr>
<tr>
<td>6</td>
<td>DMF (5 mL)</td>
<td>DMF (5 mL)/CH₃CN (15 mL)</td>
<td>1.0:1.1</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>CH₃CN (5 mL)</td>
<td>CH₃CN (5 mL)/toluene (10 mL)</td>
<td>1:9.3</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>CH₃CN (20 mL)</td>
<td>CH₃CN (20 mL)/toluene (20 mL)</td>
<td>1:5.1</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>CH₃CN (5 mL)</td>
<td>CH₃CN (5 mL)/toluene (5 mL)</td>
<td>1:7</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>CH₃CN (10 mL)</td>
<td>CH₃CN (10 mL)/toluene (10 mL)</td>
<td>0:1 (1:9)**</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>DMF (5 mL)</td>
<td>DMF(5 mL)/CH₃CN (5 mL)</td>
<td>(1:3.5)**</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>THF (5 mL)</td>
<td>THF (5 mL)/toluene (5 mL)</td>
<td>1.0:3.5</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>CH₃CN (5 mL)</td>
<td>CH₃CN (5 mL)/toluene (5 mL)</td>
<td>1.0:33.7</td>
<td>&gt;100</td>
</tr>
<tr>
<td>14</td>
<td>CH₃CN (5 mL)</td>
<td>CH₃CN (5 mL)/CH₂Cl₂ (5 mL), 0°C</td>
<td>(0:1)**</td>
<td>57</td>
</tr>
</tbody>
</table>

* Column chromatography (EtOAc/hexanes, 7:3); ** before column chromatography

**Table 5. Stereoselectivity for glycosylation of the sodium salt of 6-(2-propylimidazol-1-yl)purine**

The reaction was repeated with 21c in DMF (342 mg, 1.5 mmol) by general method 1. Volatiles were evaporated in vacuo, and the residue was chromatographed (EtOAc/hexanes ~1:1 → 7:3) to give α- (114 mg) and β-nucleoside (54 mg,
contaminated with $\alpha$-nucleoside, 1:7.3), and a mixture (321 mg, 1:1.3; 56% total, $\alpha/\beta$, 1.14:1).

**$\alpha$-Nucleoside 23:** $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.79 (s, 1H), 8.47 (s, 1H), 8.44 (s, 1H), 7.97 (d, $J$ = 8.2 Hz, 2H), 7.55 (d, $J$ = 8.2 Hz, 2H), 7.29 (d, $J$ = 8.0 Hz, 2H), 7.14 (s, 1H), 7.12 (d, $J$ = 8.0 Hz, 2H), 6.71 (dd, $J$ = 1.5, 7.0 Hz, 1H), 5.71–5.73 (m, 1H), 4.94–4.97 (m, 1H), 4.61–4.68 (m, 2H), 3.30 (t, $J$ = 7.3 Hz, 2H), 3.07–3.21 (m, 2H), 2.44 (s, 3H), 2.35 (s, 3H), 1.84 (sext, $J$ = 7.5 Hz, 2H), 1.01 (t, $J$ = 7.3 Hz, 3H).

**9-[2-Deoxy-3,5-di-O-(p-toluoyl)-$\alpha/\beta$-D-erythro-pentofuranosyl]-6-(2-hexylimidazol-1-yl)purine (24)**

<table>
<thead>
<tr>
<th>#</th>
<th>A</th>
<th>A + B</th>
<th>$\alpha/\beta$</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DMF (5 mL)</td>
<td>DMF (10 mL)</td>
<td>1.0:0.7</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>CH$_3$CN (5 mL)</td>
<td>CH$_3$CN (5 mL)/toluene (5 mL)</td>
<td>1.0:4.5-7.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(1.0:3.5-4.8)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>CH$_3$CN (10 mL)</td>
<td>CH$_3$CN (10 mL)/CH$_2$Cl$_2$ (10 mL), 0 °C</td>
<td>&lt;1.0:20</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(1.0:3.4)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>THF (5 mL)</td>
<td>THF (5 mL)/toluene (5 mL)</td>
<td>1.0:2.9</td>
<td></td>
</tr>
</tbody>
</table>

Column chromatography (MeOH/CH$_2$Cl$_2$, 1:20)

**Table 6. Stereoselectivity for glycosylation of the sodium salt of 6-(2-hexylimidazol-1-yl)purine**

The sodium salt of 21a (52 mg, 0.19 mmol) in dried CH$_3$CN (10 mL) was treated with 10 (110 mg, 0.28 mmol) in CH$_2$Cl$_2$ (10 mL) by general method 2 to give an anomeric mixture ($\alpha/\beta$ = 1:3.4). The residue was chromatographed (EtOAc/hexanes ~1:1) to give 24 (54 mg, 43%, contaminated with traces of $\alpha$-anomer, $\alpha/\beta$ ~0.5:99.5).
Recrystalization (i-PrOH) gave **24**: $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.79 (s, 1H), 8.41 (s, 1H), 8.28 (s, 1H), 7.99 (d, $J$ = 8.2 Hz, 2H), 7.88 (d, $J$ = 8.2 Hz, 2H), 7.30 (d, $J$ = 8.0 Hz, 2H), 7.20 (d, $J$ = 7.9 Hz, 2H), 7.10 (s, 1H) 6.65 (dd, $J$ = 5.8, 7.9 Hz, 1H), 5.85–5.86 (m, 1H), 4.68–4.81 (m, 3H), 3.31 (t, $J$ = 7.9 Hz, 2H), 3.18–3.21 (m, 1H), 2.93 (ddd, $J$ = 14.0, 3.7, 2.2 Hz, 1H), 2.47 (s, 3H), 2.39 (s, 3H), 1.81 (quint, $J$ = 7.3 Hz, 2H), 1.26–1.42 (m, 6H), 0.88 (t, $J$ = 7.0 Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 166.32, 166.16, 153.10, 152.01, 151.19, 148.01, 144.84, 144.48, 142.39, 130.03, 129.79, 129.53, 129.48, 128.74, 126.75, 126.53, 124.57, 120.72, 85.44, 83.52, 75.19, 64.07, 38.21, 31.78, 30.82, 29.38, 28.07, 22.76, 21.96, 21.85, 14.27; HRMS m/z 623.2982 (MH$^+$ [C$_{35}$H$_{39}$N$_6$O$_5$ = 623.2982]).

**9-[2-Deoxy-3,5-di-O-(p-toluoyl))-α/β-D-erythro-pentofuranosyl]-6-(2-dodecylimidazol-1-yl)purines (26,27)**

The sodium salt of **21b** (103 mg, 0.29 mmol) in dried CH$_3$CN (10 mL) was treated with **10** (166 mg, 0.43 mmol) in CH$_2$Cl$_2$ (10 mL) by general method 2 to give an anomeric mixture ($\alpha/\beta$ = 1:1.9). The residue was chromatographed (EtOAc/hexanes, 1:1) to give an anomeric mixture of **26** and **27** (110 mg, 51%, $\alpha/\beta$ = 1:4.3): $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 6.71 (dd, $J$ = 1.2, 6.8 Hz, 1H, H$_{1',\alpha}$), 6.63 (dd, $J$ = 6.1, 8.0 Hz, 1H, H$_{1',\beta}$). HRMS m/z 707.3906 (MH$^+$ [C$_{41}$H$_{51}$N$_6$O$_5$ = 707.3921]).
After chromatography (EtOAc/hexanes, 7:3); *(EtOAc/hexanes, 1:1); ( ), ratios before column.

<table>
<thead>
<tr>
<th>#</th>
<th>A</th>
<th>A + B</th>
<th>α/β*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Toluene (5 mL)</td>
<td>Toluene (15 mL)</td>
<td>1.0:0.4</td>
</tr>
<tr>
<td>2</td>
<td>CH₃CN (5 mL)</td>
<td>CH₃CN (5 mL)/toluene (5 mL)</td>
<td>1.0:2.7 (1:2.1)</td>
</tr>
<tr>
<td>3</td>
<td>CH₃CN (5 mL)</td>
<td>CH₃CN (15 mL)</td>
<td>1:1.2 (1.0:0.8)</td>
</tr>
<tr>
<td>4</td>
<td>DMF (5 mL)</td>
<td>DMF (5 mL)/CH₃CN (10 mL)</td>
<td>1:0.62</td>
</tr>
<tr>
<td>5</td>
<td>DMF (5 mL)</td>
<td>DMF (10 mL)</td>
<td>1:0.63</td>
</tr>
<tr>
<td>6</td>
<td>Toluene (5 mL), 85 °C</td>
<td>Toluene (15 mL), 85 °C</td>
<td>1.0:0.6</td>
</tr>
<tr>
<td>7</td>
<td>CH₃CN (10 mL)</td>
<td>CH₃CN (10 mL)/CH₂Cl₂ (10 mL), 0 °C</td>
<td>1:4.2** (1:1.9)</td>
</tr>
</tbody>
</table>

*After chromatography (EtOAc/hexanes, 7:3); **(EtOAc/hexanes, 1:1); (), ratios before column.

Table 7. Stereoselectivity for glycosylation of the sodium salt of 6-(2-dodecylimidazol-1-yl)purine

2,3,4,6-Tetra-O-benzoyl-α-D-glucopyranosyl bromide (31)

Benzoyl chloride (43.5 mL, 52.7 g, 375 mmol) was added to a solution of D-glucose (9.0 g, 50 mmol) and DMAP (1.0 g, 9.2 mmol) in dry pyridine (60 mL). The resulting solution was stirred at 60 °C overnight, and the reaction was quenched with MeOH (100 mL). Volatiles were evaporated in vacuo, and the residue was chromatographed (EtOAc/hexanes, 85:15) to give 1,2,3,4,6-penta-O-benzoyl-α-D-glucopyranose (30.7 g, 88%): ¹H NMR (300 MHz, CDCl₃) δ 4.46–4.68 (m, 3H), 5.70 (dd, J = 10.2, 3.6 Hz, 1H), 5.89 (t, J = 9.7 Hz, 1H), 6.35 (t, J = 10 Hz, 1H), 6.88 (d, J = 3.7 Hz, 1H), 7.27–8.21 (m, 25 H).
HBr/HOAc (30% w/w, 25 mL) was added to a solution of this material (1.82 g, 2.6 mmol) in dried CH$_2$Cl$_2$ (6 mL), and the solution was stirred at ambient temperature for 3 h. Volatiles were evaporated, and the residue was chromatographed (EtOAc/hexanes, 15:85) to give a solid. This material was dissolved in CH$_2$Cl$_2$ and washed (NaHCO$_3$/H$_2$O, 3 x). Volatiles were evaporated to give solid 31 (1.54 g, 90%):

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 4.51 (dd, $J = 4.6$, 12.5 Hz, 1H), 4.67 (dd, $J = 2.7$, 12.5 Hz, 1H), 4.72–4.75 (m, 1H), 5.33 (dd, $J = 4.1$, 10.9 Hz, 1H), 5.82 (t, $J = 10.1$ Hz, 1H), 6.27 (t, $J = 9.9$ Hz, 1H), 6.87 (d, $J = 4.3$ Hz, 1H), 7.29–7.59 (m, 12H), 7.88 (d, $J = 8.3$ Hz, 2H), 7.95 (d, $J = 8.3$ Hz, 2H), 8.00 (d, $J = 8.3$ Hz, 2H), 8.07 (d, $J = 8.3$ Hz, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 166.3, 165.8, 165.6, 165.4, 134.1, 133.9, 133.6, 133.5, 130.4, 130.2, 130.1, 130.0, 129.1, 128.84, 128.77, 128.74, 128.64, 87.1, 73.0, 71.7, 70.9, 68.2, 62.2; HRMS (FAB) $m/z$ 681.0741 (MNa$^+$ [C$_{34}$H$_{27}$BrO$_9$Na] = 681.0746).

9-(2,3,4,6-Tetra-O-benzoyl-β-D-glucopyranosyl)-6-(2-hexylimidazol-1-yl)purine (32)

The sodium salt of 21a (54 mg, 0.19 mmol) in dried DMF (10 mL) was treated with 31 (0.20 g, 0.3 mmol) in dried DMF (5 mL) by general method 1 for 50 h, and then at 60 °C overnight (reaction incomplete, TLC). The residue was chromatographed (25 g silica gel, MeOH/CH$_2$Cl$_2$, 1:12) to give 32 (52 mg, 31%) as the only product: $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.73 (s, 1H), 8.45 (s, 2H), 8.02 (d, $J = 8.3$ Hz, 2H), 7.95 (d, $J = 8.0$ Hz, 2H), 7.83 (d, $J = 8.3$ Hz, 2H), 7.73 (d, $J = 8.0$ Hz, 2H), 7.26–7.58 (m, 12H), 7.08 (s, 1H) 6.37 (d, $J = 9.2$ Hz, 1H), 6.15–6.22 (m, 2H), 5.95 (t, $J = 9.0$ Hz, 1H), 4.72–4.70 (m, 1H), 4.53–4.55 (m, 2H), 3.26 (t, $J = 7.8$ Hz, 2H), 1.76 (quint, $J = 7.8$ Hz, 2H), 1.27–1.43 (m, 6H), 0.86 (t, $J = 7.0$ Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 166.2, 165.8, 165.5, 165.1, 153.7, 152.4, 151.2, 148.1, 141.9, 134.1, 134.0, 133.8, 133.6, 130.2, 130.1, 130.03,
129.99, 129.5, 128.84, 128.69, 128.6, 127.8, 123.7, 120.7, 81.3, 75.9, 73.2, 71.2, 69.1, 62.7, 31.8, 30.9, 29.4, 28.0, 22.8, 14.3; HRMS m/z 871.3055 (MNa⁺)
[C₄₈H₄₄N₆O₉Na] = 871.3068).

A parallel reaction with K₂CO₃ as base gave a lower yield (33 mg, 20%).

9-(2,3,4,6-Tetra-O-benzoyl-β-D-glucopyranosyl)-2-chloro-6-(2-propylimidazol-1-yl)purine (33)

The sodium salt of 2-chloro-6-(2-propylimidazol-1-yl)purine (28) (51 mg, 0.19 mmol) in dried DMF (10 mL) was treated with 31 (0.19 g, 0.3 mmol) in dried DMF (2 mL) by general method 1. The reaction was incomplete (TLC), but cleaner than the glycosylation of 21a. The reaction mixture was diluted with CH₂Cl₂ (100 mL), and the solution was washed (H₂O) and dried (Na₂SO₄). Volatiles were evaporated in vacuo, and the residue was chromatographed successively (3 x) (25 g silica gel, MeOH/CH₂Cl₂, 1:90 → 1:40; 1:60; EtOAc/hexanes, 3:7 → 1:1) to give 33 (49 mg, 30%, contaminated with traces of an unidentified byproduct): ¹H NMR (500 MHz, CDCl₃) δ 8.53 (s, 1H), 8.44 (s, 1H), 8.03 (d, J = 7.9 Hz, 2H), 7.95 (d, J = 8.0 Hz, 2H), 7.82 (d, J = 7.9 Hz, 2H), 7.76 (d, J = 7.9 Hz, 2H), 7.26–7.58 (m, 12H), 7.06 (s, 1H), 6.34 (d, J = 9.1 Hz, 1H), 6.20 (d, J = 9.7 Hz, 1H), 6.05 (d, J = 9.5 Hz, 1H), 5.93 (d, J = 9.1 Hz, 1H), 4.54–4.71 (m, 3H), 3.2 (t, J = 7.6 Hz, 1H), 1.77 (sext, J = 7.6 Hz, 1H), 1.01 (t, J = 7.4 Hz, 1H); ¹³C NMR (125 MHz, CDCl₃) δ 166.2, 165.7, 165.3, 165.1, 155.0, 153.8, 151.4, 148.2, 142.0, 134.1, 134.0, 133.7, 133.6, 130.11, 130.08, 129.97, 129.94, 129.46, 129.14, 128.94, 128.75, 128.67, 128.60, 127.6, 122.1, 120.5, 81.2, 76.1, 73.0, 71.4, 69.0, 62.6, 33.0, 21.5, 14.1; HRMS m/z 841.2390 (MH⁺ [C₄₅H₃₈ClN₆O₉] = 841.2389).
The reaction was repeated with the sodium salt of 28 (51 mg, 0.19 mmol) in dried DMF (5 mL) and 31 (0.25 g, 0.37 mmol) in dried DMF (2 mL) at 60 °C overnight (reaction complete, TLC). The reaction mixture had two spots on TLC (MeOH/CH₂Cl₂) with almost identical UV spectra [major component: (MeOH) max 226, 284 nm, min 261 nm; minor component: max 226, 284 nm, min 262 nm]. Volatiles were concentrated in vacuo, and the residue was adsorbed on a limited amount of silica gel and chromatographed (EtOAc/hexanes, 1:1 → EtOAc) to give 31 [68 mg, 41%, contaminated with an unidentified byproduct (3.6:1)].

2-Amino-9-(2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl)-6-(imidazol-1-yl)purine (34)

The sodium salt of 2-amino-6-(imidazol-1-yl)purine (29) (51.4 mg, 0.25 mmol) in dried DMF (10 mL) was treated with 31 (0.29 g, 0.44 mmol) in dried DMF (3 mL) by general method 1. The reaction mixture was diluted with CH₂Cl₂ (100 mL), and the solution was washed (H₂O) and dried (Na₂SO₄). Volatiles were evaporated in vacuo, and the residue was chromatographed (25 g silica gel, MeOH/CH₂Cl₂, 1:90 → 1:30) to give 34 (23 mg, 11%): ¹H NMR (500 MHz, CDCl₃) δ 8.99 (s, 1H), 8.25 (s, 1H), 8.11 (s, 1H), 8.02 (d, J = 7.6 Hz, 2H), 7.95 (d, J = 7.6 Hz, 2H), 7.83 (d, J = 7.6 Hz, 2H), 7.77 (d, J = 7.6 Hz, 2H), 7.27–7.56 (m, 12H), 7.18 (s, 1H), 6.10–6.18 (m, 3H), 5.91 (t, J = 9.1 Hz, 1H), 5.01 (s, 2H), 4.47–4.72 (m, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 166.2, 165.9, 165.3, 165.1, 159.6, 155.9, 146.6, 139.2, 137.6, 133.9, 133.7, 133.5, 131.1, 130.57, 130.10, 130.07, 129.99, 129.96, 129.55, 128.74, 128.67, 128.61, 128.08, 117.6, 116.6, 80.9, 75.6, 73.3, 70.9, 69.2, 62.8; HRMS m/z 802.2246 (MNa⁺ [C₄₂H₃₃N₇O₉Na] = 802.2237).
2-Acetamido-9-(2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl)-6-(imidazol-1-yl)purine (35)

The sodium salt of 2-acetamido-6-(imidazol-1-yl)purine (30) (62 mg, 0.25 mmol) in dried DMF (10 mL) was treated with 31 (0.25 g, 0.37 mmol) in dried DMF (2 mL) by general method 1. The reaction mixture was diluted with CH$_2$Cl$_2$ (100 mL), and the solution was washed (H$_2$O) and dried (Na$_2$SO$_4$). Volatiles were evaporated in vacuo, and the residue was chromatographed twice (25 g silica gel, MeOH/CH$_2$Cl$_2$, 1:90 → 1:30; EtOAc/hexanes, 1:1 → EtOAc) to give 35 (33 mg, 16%): $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 9.04 (s, 1H), 8.31 (s, 1H), 8.28 (s, 1H), 8.01 (br, 3H), 7.96 (d, $J = 7.7$ Hz, 2H), 7.83 (d, $J = 7.6$ Hz, 2H), 7.75 (d, $J = 7.6$ Hz, 2H), 7.23–7.57 (m, 13H), 6.11–6.22 (m, 3H), 5.92 (t, $J = 9.5$ Hz, 1H), 4.53–4.73 (m, 3H), 2.60 (s, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 170.8, 166.2, 165.8, 165.3, 165.1, 154.9, 152.9, 146.4, 143.2, 141.6, 137.7, 134.1-127.8, 119.0, 117.7, 81.5, 75.9, 73.0, 71.2, 69.0, 62.7, 29.9; HRMS m/z 844.2356 (MNa$^+$) [C$_{44}$H$_{35}$N$_7$O$_{10}$Na] = 844.2343).

The reaction was repeated with the sodium salt of 30 (62 mg, 0.25 mmol) in dried DMF (5 mL) and 31 (0.25 g, 0.37 mmol) in dried DMF (2 mL) at 60 °C overnight (reaction complete, TLC). The reaction mixture had two spots on TLC (MeOH/CH$_2$Cl$_2$) with almost identical UV spectra [major component: (MeOH) max 230, 279 nm, min 255 nm; minor component: max 228, 280 nm, min 257 nm]. Volatiles were concentrated in vacuo, and the residue was adsorbed on a limited amount of silica gel and chromatographed (EtOAc/hexanes, 1:1 → EtOAc) to give 33 (64 mg, 29%) with traces of byproduct.
5. References and Notes


17. Wenzel, T. J.; Wilcox, J. D. Chirality, 2003, 15, 256-270.


Chapter 5

Synthesis of 2-Chloro-2’-deoxyadenosine

1. Introduction

2-Chloro-2’-deoxyadenosine (Cladribine, 2-CdA) is a purine 2’-deoxynucleoside analogue with a 2-chloro substituent on the purine ring. It is resistant to deamination by adenosine deaminase (ADA) due to its protonation at N7 instead of N1, which prevents addition of water and deamination at C6.1 2-CdA was shown to have antileukemic activity in 1972.2 The potential of 2-CdA as a chemotherapeutic agent against lymphoid neoplasms was proposed by Carson et al.3,4 It is lethal to resting normal lymphocytes and slowly dividing malignant T lymphocytes, as well as to proliferating lymphocytes. 2-CdA is a drug of choice for hairy cell leukemia, with ~85% complete responses.5,6 It has also been used for the treatment of several other neoplasms,7 including acute myelogenous leukemia,8 chronic lymphocytic leukemia,9 chronic myelogeneous leukemia, cutaneous T-cell lymphoma10 and non-Hodgkin’s lymphoma.11 Investigations of treatment of multiple sclerosis,12 systemic lupus erythematosis-associated glomerulonephritis and other rheumatoid and immune disorders with 2-CdA are in progress.

2-CdA is taken up by cells through nucleoside transporters in the cell membrane, and converted intracellularly to 2-CdATP, its active form, by the sequential actions of deoxycytidine kinase (dCK), AMP kinase and nucleoside diphosphate kinase.13 2-CdATP exists in dynamic equilibria of phosphorylation and dephosphorylation. The rate determining step for phosphorylation is 2-CdA → 2-CdAMP catalyzed by deoxycytidine kinase. The rate determining step for dephosphorylation is 2-CdAMP → 2-CdA catalyzed
by 5′-nucleotidase (5′NT). The high activity of dCK and low activity of 5′NT in lymphoid cells, as well as the resistance of 2-CdA to ADA deamination, cause the accumulation of 2-CdATP. 2-CdA can also be phosphorylated by a mitochondrial deoxyguanosine kinase (dGK), and 2-CdATP was reported to be trapped in mitochondrial compartments.

Knowledge concerning the mechanism of cytotoxicity of 2-CdA is multifaceted. It was proposed that 2-CdATP disrupted cell metabolism in replicating cells by its incorporation into DNA of the dividing cells, leading to chain termination. It retards DNA synthesis as an inhibitor of DNA polymerases α and β. It inhibits the enzyme ribonucleotide reductase, causing deoxynucleotide pool imbalance. This depression of the deoxynucleotide pool further facilitates the incorporation of 2-CdA into DNA (self-potentiation). It was first suggested that incorporation of 2-CdATP in resting cells accumulated DNA strand breaks. This activates poly(ADP-ribose) synthetase (PARB), and results in NAD+/ATP depletion and resulting cell necrosis (instead of the originally suggested “apoptosis”). However, recent studies have disclosed that an accumulation of DNA strand breaks caused by 2-CdA induces mitochondrial permeability transitions via p53 activation. This results in cell death irrespective of downstream events, and the PARB-mediated necrosis is usually a secondary mechanism, although occasionally it is the primary event when other killing pathways are blocked. 2-CdATP can also cooperate with cytochrome c and induce caspase-3 activation, and then the caspase proteolytic cascade causes cell apoptosis. It was also suggested that activation of caspases, as a result of mitochondrial permeability transitions, may only direct cell death via apoptosis and be incapable of induction of cell killing. Recently, perturbation
of DNA methylation and alteration of DNA conformation (particularly TATA box) by 2-CdA were observed and these might be another possible contributions to chemotherapeutic mechanisms.\textsuperscript{25a,b} The primary toxicity of the compound is myelosuppression, which is a dose-limiting factor.

Cladribine was first prepared by Venner in Fischer-Helferich syntheses of 2’-deoxyuridineosides in 1960. It was an intermediate for synthesis of both 2’-deoxyguanosine and 2’-deoxyinosine.\textsuperscript{26} Ikehara et al.\textsuperscript{27,28} also employed 2-CdA as an intermediate in the preparation of 2’-deoxyadenosine. In the latter example, 2-CdA was obtained by coupling the mercury salt of 2,8-dichloroadenine with 2-O-acetyl-3-O-tosyl-5-O-methoxycarbonyl-D-xylofuranosyl chloride, followed by indirect deoxygenation via desulfurization of 8,2’-anhydro-9-(β-D-arabinofuranosyl)-2-chloro-8-thioadenine.

As a target compound, Robins et al.\textsuperscript{2} coupled 2,6-dichloropurine with 1,3,5-tri-O-acetyl-2-deoxy-α-D-erythro-pentofuranose using the fusion method to give an anomeric mixture. Regiospecific ammonolysis of the 6-Cl group and the protecting esters gave 2-CdA and its α-isomer. This mixture was reacylated with p-toluoyl chloride and separated by chromatography. Deprotection in methanolic sodium methoxide at ambient temperature gave 2-CdA with an overall yield of 16%. Fusion of 2,6-dichloropurine with methyl 2-deoxy-3,5-di-O-(p-toluoyl)-D-erythro-pentofuranoside gave a mixture of anomers, which was separated by silica gel chromatography. Ammonolysis of the 6-Cl group and simultaneous deprotection of the p-toluoyl groups gave 2-CdA with an overall yield of 8%.

The current industrial procedure to prepare 2-CdA was devised by Robins et al.\textsuperscript{29,30} via direct glycosylation. Direct glycosylation of 2-deoxyfuranose derivatives
invariably gives anomeric mixtures, and both N7 and N9 can be glycosylated to give regioisomers as mentioned in Chapter 1. The similar mobility of these isomeric products during chromatographic separation causes difficulties for purification. Yields of the desired products are usually low. In the Robins procedure, the sodium salt of 2,6-dichloropurine was prepared in situ by treatment with NaH in CH$_3$CN at ambient temperature. This salt was coupled with 2-deoxy-3,5-di-O-(p-toluoyl)-α-D-erythro-pentofuranosyl chloride in CH$_3$CN to give a mixture of β-anomers (N9 and N7) in yields of 59% and 13%, respectively. A more detailed study by Hildebrand and Wright$^{31}$ discovered that both α- and β-anomers were formed, and yields of 50%, 15% and 1.5% were obtained for the 9-β-, 7-β- and 9-α- nucleosides, respectively. The formation of N7 coupling products resulted from the ambident character of the sodium salt of the purine. Chromatographic separation of the mixture gave 2,6-dichloro-9-[2-deoxy-3,5-di-O-(p-toluoyl)-β-D-erythro-pentofuranosyl]purine, which was ammonolyzed with methanolic ammonia at 100 °C to give 2-CdA in an overall yield of 42%.

The Robins procedure has been further modified to improve both the regioselectivity for N9 isomer and the stereoselectivity for the β-anomer by altering the purine aglycon and changing the reaction medium. Gupta and Munk$^{32}$ synthesized 2-CdA by coupling of 6-alkylamido-2-chloropurines with the 2-deoxysugar derivative. Kazimierczuk and Kaminski$^{33}$ prepared 2-CdA by condensation of 2-chloropurine derivatives (6-alkoxy, halo, alkylthio, etc.) with 2-deoxy-3,5-di-O-aroyl-α-D-erythro-pentafuranosyl chlorides (aroyl = p-chlorobenzoyl, p-bromobenzoyl, p-methoxybenzoyl, p-nitrobenzoyl, 2,4-dinitrobenzoyl) in the presence of alkali metal hydrides or hydroxides and phase-transfer catalysts, followed by ammonolysis and deprotection. Neither
procedure excluded the formation of N7-isomers. Gerszberg and Alonso\textsuperscript{34} reported a synthesis of cladribine using the sodium salt method with acetone as solvent. By controlling the reaction time, high stereoselectivity was attained by sacrificing the yield. The low yield (30% based on 2-chloroadenine) probably resulted from the low solubility of the sodium salt of 6-amino-2-chloropurine. The high regioselectivity might have been enhanced by intermolecular hydrogen bonding between the 6-amino group of the purine and acetone.

The second major method for preparation of 2-CdA is by modification of natural nucleosides. Chen\textsuperscript{35} prepared 2-CdA from guanosine in a low overall yield (2.8% for 8 steps). 2’,3’,5’-Tri-\(O\)-acetylguanosine was deoxychlorinated at C6; subsequent diazotization/chloro-dediazoniation, and ammonolysis gave 2-chloroadenosine. Protection of the 3’- and 5’-OH groups as 3’,5’-\(O\)-tetraisopropyldisiloxyl, and acylation of the 2’-OH group with phenyl chlorothionoformate, followed by radical deoxygenation with an organic tin hydride and desilylation gave 2-CdA. Robins et al.\textsuperscript{36} reported a concise synthesis of 2-CdA from the more expensive 2’-deoxyguanosine. Transformation of the 6-oxo group to 6-Cl and/or 6-\(O\)-arylsulfonyl, diazotization/chloro-dediazoniation at C2, followed by ammonolysis at C6 and simultaneous deprotection of the sugar moiety gave 2-CdA in good overall yields (64-75%).

A third method for preparation of 2-CdA employed enzymatic glycosyl transfer. Mikhailopulo et al.\textsuperscript{37} reported an enzymatic synthesis by direct transfer of the 2-deoxyfuranose moiety of thymidine to 2-chloroadenine using a trans-N-deoxyribosylase. Barai et al.\textsuperscript{38} described a similar glycosyl transfer from 2’-deoxyguanosine to 2-
chloroadenine with a large excess of 2’-deoxyguanosine, which gave a low yield (<27%) based on 2’-deoxyguanosine.

Thus, there is a great need for cost effective methods to prepare 2-CdA in high yields. A regiospecific and highly stereoselective glycosylation for synthesis of 9-(2-deoxy-β-D-erythro-pentofuranosyl)purine nucleosides has been developed in Chapter 4. The strategies of using binary solvent mixtures for glycosylation, and increase of purine solubility by introduction of lipophilic groups have been applied to the synthesis of cladribine.

2. Results and Discussion

2.1. Synthesis of 6-(2-Alkylimidazol-1-yl)-2-chloropurines

6-(2-Alkylimidazol-1-yl)-2-chloropurines were prepared as possible pharmaceutical agents and as starting materials for the synthesis of cladribine. The 2-alkylimidazoles were prepared according to Katritzky’s method as in Chapter 4. The 6-Cl group of 2,6-dichloropurine was selectively replaced by the imidazoles in DMF at 65 °C with moderate to good yields (Scheme 1). The imidazoles acted as both the reactant and base. Large excesses of the imidazoles were used to drive the reactions to completion, except for 2-dodecylimidazole (1-molar equivalent of imidazole was used with incomplete reaction). Addition of DIPEA did not enhance the yield.
Scheme 1. Synthesis of 6-(2-alkylimidazol-1-yl)-2-chloropurines (Method 1)

In the second route (as in Chapter 3), the 2-amino group of guanosine was protected with a bulky trityl group. Replacement of the 6-oxo group with 2-propylimidazole using the modified Appel method, followed by detritylation and diazotization/chloro-dediazoniation gave 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-2-chloro-6-(2-propylimidazol-1-yl)purine (31%) (Scheme 2). The relatively low yield resulted from multiple column separations to remove the Ph₃PO. Acidic deglycosylation gave 2-chloro-6-(2-propylimidazol-1-yl)purine.

Scheme 2. Synthesis of 6-(2-propylimidazol-1-yl)-2-chloropurine (Method 2)
Because of the limitations of Katritzky’s method, a series of 2-alkylimidazoles was prepared according to a different procedure (Scheme 3).\textsuperscript{39} \(\text{NH}_4\text{HCO}_3\), an aliphatic aldehyde and glyoxal in \(\text{H}_2\text{O}\) were stirred overnight to give the imidazole derivatives in excellent yields (for aldehydes with low boiling points). Evaporation of volatiles gave nearly \(^1\text{H}\) NMR-pure products. With less volatile aldehydes, such as 3-phenylbutyraldehyde, chromatographic separation gave lower yields. An even lower yield for \(\text{PhCH}_2\text{CHO}\) is due to both chromatographic separation losses and side reactions of alternative cyclization to give 3,5-diphenylpyridine (Scheme 4).\textsuperscript{40} Synthesis of 2-benzylimidazole via Katritzky’s method was unsuccessful due to the difficulty of cleavage of the N1 protecting group.

![Scheme 3. Synthesis of 2-alkylimidazoles by cyclization](image-url)
Scheme 4. Competitive pathways for cyclizations of PhCH₂CHO

Scheme 5. Synthesis of 2-chloro-6-(imidazol-1-yl)purines (Method 3)

The 2-chloropurine derivatives also were prepared by displacement of the 6-Cl group from 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-2,6-dichloropurine (as in Chapter 3, 10). Treatment of 10 with excess 2-alkylimidazoles in CH₃CN at 65 °C followed by acidic deglycosylation gave the 2-chloro-6-(2-alkylimidazol-1-yl)purines in moderate to excellent yields (Scheme 5). The optimized yield for the 3 steps for 2-chloro-6-(2-propylimidazol-1-yl)purine was 82%.
2.2. Synthesis of cladribine

The 2-chloro-6-(2-alkylimidazol-1-yl)purines were coupled with 2-deoxy-3,5-di-\(O\)-(\(p\)-toluoyl)-\(\alpha\)-\(D\)-erythro-pentofuranosyl chloride using the sodium salt method in a binary solvent mixture (Scheme 6). The \(d_r\) was measured by 500 MHz \(^1\)H NMR as shown in Figures 1 and 2. High stereoselectivity was usually achieved (Figure 2). An optimized yield of 95% was obtained for 2-chloro-6-(2-propylimidazol-1-yl)purine. Coupling of 2-chloro-6-(imidazol-1-yl)purine and the chlorosugar gave only moderate stereoselectivity (\(\beta/\alpha \sim 1.85:1\)) in the same solvent mixtures, which might be due to the more polar local reaction environment and low solubility of the purine salt.

Regiospecific glycosylation of 2-chloro-(4,5-diphenylimidazol-1-yl)purine was observed, which suggested that the regioselectivity was strongly affected by the size and/or reactivity of the electrophile. Alkylation of the sodium salt of 2-chloro-(4,5-diphenylimidazol-1-yl)purine with EtI gave both N9 and N7 regioisomers (as observed in Chapter 3). The bulky chlorosugar gave only the N9 regioisomer.

![Figure 1. Determination of the \(d_r\) for glycosylation of 2-chloro-6-(2-propylimidazol-1-yl)purine (3a) in \(\text{CH}_3\text{CN}/\text{CH}_2\text{Cl}_2\) in comparison with that of 2-chloro-6-(2-isopropylimidazol-1-yl)purine (3d) in DMF](image)
Figure 2. Effects of 6-(2-alkyl/4,5-diphenylimidazol-1-yl) substituents on the stereoselectivity for glycosylation of 2-chloropurines
Scheme 6. Stereoselectivity for glycosylation of the sodium salts of 2-chloro-6-(imidazol-1-yl)purines

Ammonolysis of the 6-imidazolyl groups in methanolic NH$_3$ was incomplete at 100 °C overnight. Mixtures of the ammonolized (and deprotected) products: UV (MeOH) max 261–265 nm; and some 2-chloro-6-(imidazol-1-yl)nucleosides: UV (MeOH) max 280–285 nm were obtained. Ammonolysis of 2-acetamido-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-6-(imidazo-1-yl)purine at ambient temperature gave 2-amino-6-(imidazol-1-yl)-9-(β-D-ribofuranosyl)purine (14) in good yield. Therefore, activation of the imidazole group for SNAr displacement was studied. Activation with Lewis acids to enhance the leaving group ability was tried first. 2-Chloro-9-[2-deoxy-3,5-di-O-(p-toluoyl)-β-D-erythro-pentofuranosyl]-6-(2-propylimidazol-1-yl)purine was treated with
BF$_3$·Et$_2$O in CH$_2$Cl$_2$. Formation of the Lewis acid-base complex was complete within 15 min. Volatiles were evaporated, and the solid was transferred to a pressure tube. NH$_3$/MeOH (26%) was added, and the sealed solution was stirred at 60 °C. However, the displacement was incomplete even after several days. 2-Chloro-9-[2-deoxy-3,5-di-O-(p-toluoyl)-β-D-erythro-pentofuranosyl]-6-(2-propylimidazol-1-yl)purine was then treated with BH$_3$·DMS in CH$_2$Cl$_2$. Formation of the Lewis acid-base complex was complete within 15 min. Volatiles were evaporated, and the solid was transferred to a pressure tube. NH$_3$/MeOH (26%) was added, and the sealed solution was stirred at 60 °C. The displacement was complete, but a new byproduct was observed [UV (MeOH) max 263 nm, broad], which migrated faster (TLC) than the product spot [UV (MeOH) max 265 nm].

Attempts at activation of the imidazole ring via methylation gave incomplete reactions, as previous results in our lab had indicated. The more reactive benzyl iodide was tried at elevated temperature. Benzylation of 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-2-chloro-6-(2-propylimidazol-1-yl)purine followed by ammonolysis gave 6-amino-2-chloro-9-(β-D-ribofuranosyl)purine with an overall yield of 50% after recrystallization (EtOH). Both the benzylation and ammonolysis reactions were clean and gave only one product (TLC) (Scheme 7).

This strategy was applied to the synthesis of cladribine. Benzylation was complete, except with the sterically hindered imidazole compounds such as 12d and 12i. Benzylation of 12h was complete, but darkening of the reaction mixture due to decomposition was observed (Scheme 8). Displacement of 3-benzylimidazole from C6 and concomitant deprotection in methanolic ammonia at 60 °C gave clean products with
moderate to excellent yields. An optimized yield of 83% for 2 steps with displacement/deprotection of 12a was obtained.

Scheme 7. Activation of the imidazole ring as a better leaving group by benzylation at N3

<table>
<thead>
<tr>
<th>R</th>
<th>a%/#</th>
<th>b%*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Propyl (a)</td>
<td>&gt;100</td>
<td>83</td>
</tr>
<tr>
<td>2-Isopropyl (d)</td>
<td>incomplete</td>
<td></td>
</tr>
<tr>
<td>2-Butyl (e)</td>
<td>&gt;100</td>
<td>85</td>
</tr>
<tr>
<td>2-Pentyl (f)</td>
<td>77**</td>
<td>73</td>
</tr>
<tr>
<td>2-(2-Phenylpropyl) (g)</td>
<td>71**</td>
<td>43</td>
</tr>
<tr>
<td>2-Benzyl (h)</td>
<td>complete/</td>
<td></td>
</tr>
<tr>
<td></td>
<td>decomposition</td>
<td></td>
</tr>
<tr>
<td>4,5-Diphenyl (i)</td>
<td>incomplete</td>
<td></td>
</tr>
</tbody>
</table>

#crude yield; *for two steps; **impure starting material

Scheme 8. Synthesis of 2-chloro-2'-deoxyadenosine
6-Amino-2-chloropurine was prepared by displacement of the 6-Cl group of 2,6-dichloropurine in methanoic ammonia at 100 °C (87%). Glycosylation of 6-amino-2-chloropurine in the binary solvent mixture (CH$_3$CN/toluene) by the sodium salt procedure gave a low de (54%) in excellent yield (Scheme 9). Successive chromatography (MeOH/CH$_2$Cl$_2$, 1:12; EtOAc/hexanes, 3:7 → 85:15) gave the purified β-anomer (46%) plus an anomic mixture (36%).

![Chemical structure image](image)

Scheme 9. Stereoselectivity for glycosylation of the sodium salt of 6-amino-2-chloropurine

NOE effects have been applied for the identification of anomic configurations of 2’-deoxynucleosides.\(^1\) In both the S- and N-type conformations of the β-anomers, H1’ and H4’ are in spatial proximity and on the same side of the furanose ring (Figure 3). H1’ and H3’ are on opposite faces of the furanose ring. When H1’ was irradiated, an
enhancement in the signals for H4' (small), H8 and H2',2'' were observed. This confirmed the configuration of the β-anomer.

![Diagram of β-anomer](Image)

**Figure 3. NOE effects for 6-amino-2-chloro-9-[2-deoxy-3,5-di-O-(p-toluoyl)-β-D-erythro-pentofuranosyl]purine (20)**

In an α-anomer, H1’ and H4’ are on opposite sides of the furanose ring (Figure 4). H1’ and H3’ are on the same side, and the relative distance between H1’ and H3’ depends on the conformation of the furanose ring. For the S-type conformation (\( \alpha_S \)) of an α-anomer, H1’ and H3’ have a larger separation. For the N-type conformation (\( \alpha_N \)) of an α-anomer, H1’ and H3’ are closer. When H3’ was saturated, enhancements of the signals for H4’ and H2’,2’’ were observed; and no enhancement of the signal for H1’ was
noted. When H1’ was irradiated, enhancements of the H8 and H2’,2’’ signals were observed, but not for the H4’ or H3’ signals. This confirmed the $\alpha$-anomeric structure.

Figure 4. NOE effects for compound 21
2-Chloro-9-[2-deoxy-3,5-di-O-(p-toluoyl)-β-D-erythro-pentofuranosyl]-6-(2-propylimidazol-1-yl)purine gave similar NOE difference spectra to that of 6-amino-2-chloro-9-[2-deoxy-3,5-di-O-(p-toluoyl)-β-D-erythro-pentofuranosyl]purine (Figure 5). The absolute configuration was confirmed by X-ray crystal data for the final product cladribine (Figure 6). 42

**Figure 5.** NOE effects for 2-chloro-9-[2-deoxy-3,5-di-O-(p-toluoyl)-β-D-erythro-pentofuranosyl]-6-(2-propylimidazol-1-yl)purine (12a)

**Figure 6.** ORTEP diagram of cladribine
2.3. Biological assays

2-Chloro-6-(2-propylimidazol-1-yl)purine showed antitumor activity against human T-lymphocyte cells (Molt4/C8, CEM/0) (IC\(_{50}\) = 9.1 ± 2.2 µM; IC\(_{50}\) = 9.6 ± 1.5 µM). Further modification of 2-chloro-6-(imidazol-1-yl)purine may enhance its antitumor/antiviral activity and lower its cytotoxicity. 2-Chloro-6-(imidazol-1-yl)purine derivatives were evaluated against cytomegalovirus in HEL cell cultures and HIV-1 and HIV-2 in human T-lymphocyte (CEM) cells by Professor E. De Clercq. Most of these 2-chloro-6-(imidazol-1-yl)purine derivatives have moderate inhibition activity against cytomegalovirus, and 2-chloro-9-ethyl-6-(4,6-diphenylimidazol-1-yl)purine showed significant activity (EC\(_{50}\) = 1.4 µM against AD-169 strain and EC\(_{50}\) = 1.0 µM against Davis strain) with relatively low cytotoxicity (MCC ≥ 16 µM) in comparison to Gancilovir (EC\(_{50}\) = 5.1 µM against AD-169 strain, EC\(_{50}\) = 2.5 µM against Davis strain and MCC > 1400 µM) and Cidofovir (EC\(_{50}\) = 0.47 µM against AD-169 strain, EC\(_{50}\) = 0.79 µM against Davis strain and MCC > 1400 µM). 2-Chloro-6-(2-pentylimidazol-1-yl)purine showed significant activity against TK\(^+\) varicella-zoster virus (EC\(_{50}\) = 1.9 µM against OKA strain) with relatively low cytotoxicity (MCC = 16 µM) in comparison to Acyclovir (EC\(_{50}\) = 2.3 µM and MCC > 1500 µM) and Brivudin (EC\(_{50}\) = 0.029 µM and MCC = 1201 µM). These 2-chloro-6-(imidazol-1-yl)purine derivatives also showed weak antiviral activity against HIV-1 and HIV-2.

3. Conclusions

Cladribine was prepared with an overall yield of 48% from inexpensive guanosine in 8 steps and 57% from 2,6-dichloropurine in 4 steps. 2-Chloro-6-(2-propylimidazol-1-yl)purine was prepared either from guanosine (61% for 5 steps) or from 2,6-
dichloropurine (72%). Coupling of the substituted purine base with 2-deoxy-3,5-di-O-(p-toluoyl)-α-D-erythro-pentofuranosyl chloride in a binary solvent mixture, followed by activation of the imidazole moiety by benzylation and then ammonolysis gave cladribine in high yield (79% for 3 steps). Analogues of the 2-propylimidazole-substituted base with other lipophilic groups (butyl, pentyl and 2-phenylpropyl) worked essentially as well.

4. Experimental Section

2-Alkylimidazoles (2a, d-h)

General procedure: The procedure was analogous to a reported procedure. To a suspension of NH$_4$HCO$_3$ (16.45 g, 208.1 mmol) in H$_2$O (10 mL) was added the respective aldehyde (104 mmol), and glyoxal/H$_2$O (40% w/w, 11.9 mL, 15.09 g, 104.0 mmol). The mixture was stirred at ambient temperature overnight, and volatiles were evaporated. The residue was extracted with THF. Volatiles were evaporated to give crude material, which was chromatographed to remove imidazole formed by cyclization of contaminating formaldehyde in the glyoxal solution.

2-Propylimidazole (2a)

Treatment of butanal (9.2 mL, 7.52 g, 104 mmol) by the general procedure and evaporation of volatiles gave crude material (11 g, 96%), which was chromatographed (CH$_2$Cl$_2$ → MeOH/CH$_2$Cl$_2$, 1:60 → 1:30) to give compound 2a (7.45 g, 65%): $^1$H NMR (500 MHz, CDCl$_3$) δ 11.50 (s, 1H), 6.96 (s, 2H), 2.72 (t, $J = 7.4$ Hz, 2H), 1.77 (sext, $J =$ 7.4 Hz, 2H), 0.98 (t, $J = 7.4$ Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 149.1, 121.4, 30.7, 22.3, 14.0.
2-Isopropylimidazole (2d)

Treatment of 2-methylpropanal (9.5 mL, 7.54 g, 104 mmol) by the general procedure and evaporation of volatiles gave a crude material (10.6 g, 92%), which was chromatographed (CH$_2$Cl$_2$ → MeOH/CH$_2$Cl$_2$, 1:60 → 1:30) to give 2d (7.98 g, 70%): $^1$H NMR (500 MHz, CDCl$_3$) δ 9.70 (br, 1H), 6.96 (s, 2H), 3.10 (sept, $J = 7.0$ Hz, 1H), 1.35 (d, $J = 7.0$ Hz, 6H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 153.9, 121.4, 28.5, 22.0; HRMS m/z 110.0859 (M$^+$ [C$_6$H$_{10}$N$_2$] = 110.0844).

2-Butylimidazole (2e)

Method 1. Compound 2e was prepared by Katrizky’s method. Yield (0.444 g, 30%): LRMS m/z 125 (MH$^+$ [C$_7$H$_{13}$N$_2$] = 125), 81 (M – 43 [C$_4$H$_5$N$_2$] = 81).

Method 2. Treatment of valeraldehyde (11.1 mL, 8.99 g, 104 mmol) by the general procedure and evaporation of volatiles gave a crude material (12.0 g, 94%), which was chromatographed (CH$_2$Cl$_2$ → MeOH/CH$_2$Cl$_2$, 1:60 → 1:30) to give 2e (6.80 g, 53%): $^1$H NMR (500 MHz, CDCl$_3$) δ 10.45 (br s, 1H), 6.96 (s, 2H), 2.75 (t, $J = 7.6$ Hz, 2H), 1.71 (quint, $J = 7.6$ Hz, 2H), 1.37 (sext, $J = 7.6$ Hz, 2H), 0.90 (t, $J = 7.6$ Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 149.1, 121.5, 30.9, 28.5, 22.6, 14.0; HRMS m/z 124.1000 (M$^+$ [C$_7$H$_{12}$N$_2$] = 124.1000).

2-Pentylimidazole (2f)

Treatment of hexanal (12.5 mL, 10.43 g, 104 mmol) by the general procedure and evaporation of volatiles gave a crude material (13.5 g, 94%), which was chromatographed (CH$_2$Cl$_2$ → MeOH/CH$_2$Cl$_2$, 1:60 → 1:30) to give 2f (6.34 g, 44%): $^1$H NMR (500 MHz, CDCl$_3$) δ 10.65 (br s, 1H), 6.95 (s, 2H), 2.74 (t, $J = 7.6$ Hz, 2H), 1.73
(quint, $J = 7.6$ Hz, 2H), 1.29–1.34 (m, 4H), 0.86 (t, $J = 7.4$ Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$149.2, 121.4, 31.7, 28.8, 28.6, 22.6, 14.1; HRMS $m/z$ 138.1156 ($M^+ [C_8H_{14}N_2] = 138.1157$).

2-(2-Phenylpropyl)imidazole (2g)

Treatment of 3-phenylbutanal (15 mL, 14.96 g, 101 mmol) by the general procedure and evaporation of volatiles gave crude material, which was chromatographed (CH$_2$Cl$_2$ $\rightarrow$ MeOH/CH$_2$Cl$_2$, 1:60 $\rightarrow$ 1:30) to give an enantiomeric mixture of 2g (4.76 g, 25%): $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.18–7.33 (m, 5H), 6.88 (s, 2H), 3.22 (sext, $J = 6.8$ Hz, 1H), 2.97–3.07 (m, 2H), 1.32 (d, $J = 6.7$ Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 147.3, 146.3, 128.9, 127.1, 126.8, 39.9, 37.8, 21.9; HRMS $m/z$ 186.1145 ($M^+ [C_{12}H_{14}N_2] = 186.1157$).

2-Benzylimidazole (2h)

Method 1. 1-[(Dimethylamino)methyl]imidazole (10.1 g, 81 mmol) was dissolved in dried THF (320 mL), and cooled to -78 °C under N$_2$. 1.6 M butyllithium/hexanes (53 mL) was added dropwise, and the resulting solution was stirred at -78 °C for 1.5 h. Benzyl iodide [Prepared from BnCl (15 mL, 16.5 g, 130 mmol) and NaI (4.9 g, 45 mmol)] in THF (200 mL) was added at -78 °C. The reaction mixture was stirred overnight (-78 °C to r.t.). However, deprotection with 2 N HCl/H$_2$O was unsuccessful, and 2-benzyl-1-[(dimethylamino)methyl]imidazole was isolated: $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.25 (s, 1H), 7.45–7.66 (m, 5H), 7.15 (s, 1H), 6.64 (s, 2H), 5.13 (s, 2H), 3.23 (s, 6H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 140.6, 133.4, 131.6, 131.2, 129.8, 126.0, 121.8, 70.1, 65.4, 47.2; LRMS (EI) $m/z$ 171 ($M – 44 [C_{11}H_{11}N_2] = 171$).
Method 2. A mixture of NH₄HCO₃ (16.5 g, 208.1 mmol), H₂O (10 mL), glyoxal/H₂O (40% w/w, 12 mL, 15.2 g, 104.6 mmol) and 2-phenylethanal (11.6 mL, 12.47 g, 104 mmol) was stirred for 10 min. A sticky layer was observed, and THF (30 mL) was added. This mixture was stirred overnight at ambient temperature. The reaction was incomplete, and two major UV active products (TLC) were formed. Volatiles were evaporated in vacuo. The residue was chromatographed (EtOAc/hexanes, 15:85) to give an almost pure sample of the 3,5-diphenylpyridine byproduct (9) after washing with hexanes. Recrystalization from EtOAc gave plate-shaped crystals of 9: ¹H NMR (500 MHz, CDCl₃) δ 8.83 (d, J = 2 Hz, 2H), 8.06 (t, J = 2 Hz, 1H), 7.43–7.67 (m, 10H); ¹³C NMR (125 MHz, CDCl₃) δ 147.3, 138.0, 136.9, 133.1, 129.4, 128.5, 127.5; HRMS m/z 232.1110 (MH⁺ [C₁₇H₁₄N] = 232.1126). The other UV-absorbing product was not identified. Aqueous KMnO₄ detected another spot (UV inactive), which was eluted with MeOH/CH₂Cl₂ (1:20) to give 2h (2.55 g, 16%): ¹H NMR (500 MHz, CDCl₃) δ 7.24–4.35 (m, 5H), 6.96 (s, 2H), 4.12 (s, 2H); ¹³C NMR (125 MHz, CDCl₃) δ 147.0, 137.2, 128.9, 127.1, 35.2; HRMS m/z 158.0844 (M⁺ [C₁₀H₁₀N₂] = 158.0844).

2-Chloro-6-(2-propylimidazol-1-yl)purine (3a)

Method 1. 2,6-Dichloropurine (1) (0.38 g, 2 mmol) and 2a (1.32 g, 12 mmol) were dissolved in freshly distilled DMF (10 mL), and the mixture was stirred at 65 °C for 20 h. Volatiles were evaporated in vacuo, and the residue was dissolved in NaOH/H₂O/CH₂Cl₂ (100 mL/50 mL). The organic phase was extracted with 0.1 N NaOH/H₂O (3 x 50 mL). The combined aqueous phase was washed with CH₂Cl₂ (2 x 50 mL) and neutralized with CO₂. The precipitated solid was filtered and washed (H₂O) to give 3a (0.38 g, 72%): mp 224.5-225 °C; UV (MeOH) max 215, 288 nm (ε 25 800, 16 700), min 241 nm (ε 4500);
\(^1\)H NMR (500 MHz, DMSO-\(d_6\)) \(\delta\) 14.04 (br s, 1H), 8.69 (s, 1H), 8.43 (s, 1H), 7.06 (s, 1H), 3.12 (t, \(J = 7.5\) Hz, 2H), 1.72 (sext, \(J = 7.3\) Hz, 2H), 0.95 (t, \(J = 7.3\) Hz, 3H); \(^{13}\)C NMR (125 MHz, DMSO-\(d_6\)) \(\delta\) 157.3, 151.7, 150.2, 147.2, 146.6, 128.9, 122.5, 121.1, 32.4, 21.5, 14.5; HRMS \(m/z\) 262.0723 (\(M^+\) \([C_{11}H_{11}ClN_6] = 262.0734\)). Anal. Calcd for \(C_{11}H_{11}ClN_6\): C, 50.29; H, 4.22; N, 31.99. Found: C, 50.02; H, 4.28; N, 31.64.

Method 2. 9-(2,3,5-Tri-\(O\)-acetyl-\(\beta\)-D-ribofuranosyl)-2-chloro-6-(2-propylimidazol-1-yl)purine (5) (4.99 g, 9.6 mmol) was dissolved in HOAc (400 mL). To the solution was added AcCl (4.0 mL, 4.42 g, 56.3 mmol), and the mixture was stirred at 65 °C for 1.5 h in a sealed flask (reaction almost complete, TLC). Volatiles were evaporated in vacuo. The residue was washed (CH\(_2\)Cl\(_2\)) and dissolved in 0.1 N NaOH/H\(_2\)O. Precipitation with CO\(_2\) gave a solid (2.20 g, 88%). Recrystallization (MeOH) gave 3a (1.93 g, 77%) with identical properties to the product from Method 1.

2-Chloro-6-(2-hexylimidazol-1-yl)purine (3b)

A sample of 2,6-dichloropurine (1) (0.19 g, 1 mmol) and 2b (0.97 g, 6.36 mmol) were dissolved in freshly distilled DMF (20 mL), and the mixture was stirred at 65 °C for ~20 h (reaction incomplete, TLC). Volatiles were evaporated in vacuo, and the residue was dissolved in HOAc (5 mL), and volatiles were evaporated. The residue was chromatographed (MeOH/CH\(_2\)Cl\(_2\), 1:30) to give a solid contaminated with both starting materials. This solid was washed thoroughly with CH\(_2\)Cl\(_2\), then saturated NaHCO\(_3\)/H\(_2\)O to give 3b (0.17 g, 56%): mp 192-193 °C; UV(MeOH) max 214, 288 nm (\(\varepsilon\) 25 900, 41 200), min 240 nm (\(\varepsilon\) 4500); \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) \(\delta\) 14.03 (br, 1H), 8.70 (s, 1H), 8.42 (s, 1H), 7.06 (s, 1H), 3.16 (t, \(J = 7.7\) Hz, 2H), 1.69 (quint, \(J = 7.3\) Hz, 2H), 1.39–1.33 (m, 2H), 1.22–1.30 (m, 4H), 0.84 (t, \(J = 7.0\) Hz, 3H); \(^{13}\)C NMR (500 MHz,
DMSO-d$_6$ δ 157.3, 151.7, 150.3, 147.2, 146.7, 128.9, 122.6, 121.1, 31.7, 31.4, 30.4, 29.2, 28.2, 22.7; HRMS m/z 304.1185 (M$^+$ [C$_{14}$H$_{17}$ClN$_6$] = 304.1203).

2-Chloro-6-(2-dodecylimidazol-1-yl)purine (3c)

A mixture of 1 (50 mg, 0.27 mmol) and 2c (375 mg, 1.59 mmol) in DMF (5 mL) was stirred under N$_2$ at 65 °C for 20 h, and volatiles were evaporated in vacuo. The residue was chromatographed (MeOH/CH$_2$Cl$_2$, 1:20) to give a solid, which was recrystallized (EtOAc). The crystals were suspended in HOAc (2 mL), and volatiles were evaporated in vacuo. The solid was chromatographed (MeOH/CH$_2$Cl$_2$, 1:30) to give 3c (22 mg, 21%): $^1$H NMR (300 MHz, MeOD/CDCl$_3$) δ 8.52 (s, 1H), 8.19 (s, 1H), 7.07 (s, 1H), 3.28 (t, J = 7.8 Hz, 2H), 1.77 (quint, J = 7.8 Hz, 2H), 1.23–1.43 (m, 18 H), 0.86 (t, J = 6.6 Hz, 3H); $^{13}$C NMR (75 MHz, MeOD/CDCl$_3$) δ 156.1, 153.0, 151.7, 147.7, 143.7, 128.3, 122.3, 120.7, 32.1, 30.8, 29.84, 29.80, 29.72, 29.59, 29.56, 28.4, 22.9, 14.3; HRMS (EI) m/z 388.2139 (M$^+$ [C$_{20}$H$_{29}$ClN$_6$] = 388.2142).

2-Chloro-6-(2-isopropylimidazol-1-yl)purine (3d)

9-(2,3,5-Tri-O-acetyl-$eta$-D-ribofuranosyl)-2-chloro-6-(2-isopropylimidazol-1-yl)purine (11d) (2.96 g, 5.7 mmol, contaminated with 2d) was dissolved in HOAc (190 mL). To the solution was added AcCl (1.9 mL, 2.10 g, 26.7 mmol), and the mixture was stirred at 65 °C for 20 h in a sealed flask (reaction was complete, TLC). Volatiles were evaporated in vacuo, and the residue was washed (CH$_2$Cl$_2$) and dissolved in 0.1 N NaOH/H$_2$O (200 mL). Precipitation with CO$_2$ gave 3d (0.675 g, 54%). This solid was washed (boiling MeOH/i-PrOH) to give 3d (0.60 g, 48%): mp 268-268.5 °C; UV (MeOH) max 213, 254, 288 nm (ε 26 100, 4600, 13 100), min 239, 257 nm (ε 3700,
$^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 14.06 (s, 1H), 8.71 (s, 1H), 8.36 (s, 1H), 7.07 (d, $J = 1.6$ Hz, 1H), 3.93 (br s, 1H), 1.29 (d, $J = 6.8$ Hz, 6H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 156.4, 154.3, 151.0, 146.5, 145.8, 127.9, 122.0, 120.4, 27.7, 21.6; HRMS $m/z$ 285.0626 (MNa$^+$ [ C$_{11}$H$_{11}$ClN$_6$Na] = 285.0631). Anal. Calcd for C$_{11}$H$_{11}$ClN$_6$: C, 50.29; H, 4.22; N, 31.99. Found: C, 50.12; H, 4.27; N, 32.16.

6-(2-Butylimidazol-1-yl)-2-chloropurine (3e)

Crude 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-6-(2-butylimidazol-1-yl)-2-chloropurine (11e) (2.16 g, 4 mmol) was dissolved in acetic acid (167 mL). To the solution was added AcCl (1.67 mL, 1.84 g, 23.5 mmol), and the mixture was stirred at 65 °C for 23 h in a sealed flask (reaction complete, TLC). Volatiles were evaporated in vacuo, and the residue was washed (CH$_2$Cl$_2$) and dissolved in 0.1 N NaOH/H$_2$O (130 mL). Precipitation with CO$_2$ gave a solid (0.76 g, 57%). Recrystallization (MeOH) gave 3e (0.58 g, 44%): mp 247-247.5 °C; UV (MeOH) max 214, 254, 288 nm ($\varepsilon$ 25 600, 4700, 13 900), min 239, 257 nm ($\varepsilon$ 3800, 4600); $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 14.05 (s, 1H), 8.71 (s, 1H), 8.44 (s, 1H), 7.07 (d, $J = 1.5$ Hz, 1H), 3.17 (t, $J = 7.7$ Hz, 2H), 1.70 (quint, $J = 7.6$ Hz, 2H), 1.39 (sext, $J = 7.6$ Hz, 2H), 0.91 (t, $J = 7.4$ Hz, 3H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 156.3, 151.0, 149.5, 146.4, 145.7, 128.1, 121.7, 120.3, 29.5, 29.3, 21.9, 13.6; HRMS $m/z$ 277.0973 (MNa$^+$ [ C$_{12}$H$_{14}$ClN$_6$Na] = 277.0968). Anal. Calcd for C$_{12}$H$_{14}$ClN$_6$: C, 52.08; H, 4.74; N, 30.37. Found: C, 51.96; H, 4.85; N, 30.52.

2-Chloro-6-(2-pentylimidazol-1-yl)purine (3f)

Crude 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-2-chloro-6-(2-pentylimidazol-1-yl)purine (11f) (1.82 g, 3.3 mmol) was dissolved in HOAc (136 mL). To the solution was added AcCl (1.4 mL, 1.55 g, 19.7 mmol), and the mixture was stirred at 65 °C for 23 h in
a sealed flask (reaction complete, TLC). Volatiles were evaporated in vacuo, and the residue was washed (CH₂Cl₂) and dissolved in 0.1 N NaOH/H₂O (60 mL). Precipitation with CO₂ gave a solid (0.34 g, 48%). Recrystallization (MeOH) gave 3f (0.27 g, 38%): mp 254.5-255 °C; UV (MeOH) max 214, 254, 288 nm (ε 27 200, 13 500, 4600), min 240, 258 nm (ε 3800, 4600); ¹H NMR (500 MHz, DMSO-d₆) δ 14.05 (s, 1H), 8.70 (s, 1H), 8.44 (s, 1H), 7.07 (d, J = 1.5 Hz, 1H), 3.15 (t, J = 7.6 Hz, 2H), 1.71 (quint, J = 7.5 Hz, 2H), 1.29–1.38 (m, 4H), 0.86 (t, J = 7.2 Hz, 3H); ¹³C NMR (125 MHz, DMSO-d₆) δ 156.2, 151.0, 149.5, 146.4, 145.7, 128.1, 121.7, 120.3, 31.0, 29.6, 27.1, 21.8, 13.8; HRMS m/z 291.1138 (MNa⁺ [C₁₃H₁₆ClN₆] = 291.1125). Anal. Calcd for C₁₃H₁₅ClN₆: C, 53.70; H, 5.22; N, 28.90. Found: C, 53.55; H, 5.22; N, 29.00.

2-Chloro-6-[2-(2-phenylpropyl)imidazol-1-yl]purine (3g)

A mixture of 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-2,6-dichloropurine (0.98 g, 2.19 mmol) and 2g (4.07 g, 21.9 mmol) in CH₃CN (20 mL) was stirred at 65 °C for 17 h (reaction complete, TLC). Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH₂Cl₂, 1:90) to give a mixture of diastereomers (quantitative, contaminated with 2g). The mixture was dissolved in HOAc (91 mL), and to the solution was added AcCl (0.92 mL, 1.00 g, 12.8 mmol). The mixture was stirred at 65 °C for 25.5 h in a sealed flask (reaction complete, TLC). Volatiles were evaporated in vacuo, and the residue was dissolved in 0.1 N NaOH/H₂O (300 mL) and CHCl₃ (150 mL). The binary mixture was stirred for 2 h, and then neutralized with CO₂. The organic phase was separated, and the aqueous phase was extracted with CH₂Cl₂. The combined organic phase was dried (Na₂SO₄) and concentrated to dryness. The residue was washed (H₂O), suspended in EtOH, and filtered to give 3g (0.46 g, 62%) of material. The mother liquor
was evaporated to dryness, and the residue was chromatographed \((\text{MeOH/CH}_2\text{Cl}_2, 1:30 \rightarrow 1:12)\) to give a solid, which was washed \((\text{H}_2\text{O})\) to give the second crop \((0.18 \text{ g}, 86\% \text{ total})\). The combined solids were dissolved in \(0.1 \text{ N NaOH/H}_2\text{O (300 mL)}\).

Precipitation with \(\text{CO}_2\) gave \(3\text{g}\) as an enantiomeric mixture \((0.59 \text{ g, 80\%})\): mp 258.5-259 °C; UV \((\text{MeOH})\) max 254, 289 nm \((\varepsilon 12\ 000, 4100)\), min 240, 256 nm \((\varepsilon 3600, 4100)\); \(^1\text{H} \) NMR \((500 \text{ MHz, DMSO-}d_6\) \(\delta 14.00 \text{ (s, 1 H), 8.68 \text{ (s, 1 H), 8.35 \text{ (s, 1 H), 7.13-7.14 \text{ (m, 4 H), 7.07 \text{ (d, J = 1.9 Hz, 1 H), 7.00-7.04 \text{ (m, 1 H), 3.58 \text{ (dd, J = 14.4, 6.7 Hz, 1 H), 3.43 \text{ (dd, J = 14.2, 7.7 Hz, 1 H), 3.30 \text{ (sext, J = 7.0 Hz, 1 H), 1.23 \text{ (1.22 \text{ (s, 3 H)\)}}\))}}\); \(^{13}\text{C} \) NMR \((125 \text{ MHz, DMSO-}d_6\) \(\delta 156.4, 150.9, 147.9, 146.4, 145.8, 145.7, 128.2, 127.9, 126.6, 125.7, 121.7, 120.5, 38.3, 37.7, 21.0; \text{HRMS m/z} 361.0935 (\text{MNa}^+ [\text{C}_{17}\text{H}_{15}\text{ClN}_6\text{Na}]) = 361.0944)\). Anal. Calcd for \(\text{C}_{17}\text{H}_{15}\text{ClN}_6\): C, 60.27; H, 4.46; N, 24.81. Found: C, 60.12; H, 4.60; N, 24.66.

6-(2-Benzylimidazol-1-yl)-2-chloropurine (3h)

9-(2,3,5-Tri-\(\text{O-}\)acetyl-\(\beta\)-\(\text{D-}\)ribofuranosyl)-6-(2-benzylimidazol-1-yl)-2-chloropurine \((11\text{h})\) \((1.41 \text{ g, 2.4 mmol})\) was dissolved in HOAc \((100 \text{ mL})\). To the solution was added AcCl \((1.0 \text{ mL, 1.08 g, 13.8 mmol})\), and the mixture was stirred at 65 °C for 24 h in a sealed flask (reaction complete, TLC). Volatiles were evaporated in vacuo, and the residue was washed \((\text{CH}_2\text{Cl}_2)\) and dissolved in \(0.1 \text{ N NaOH/H}_2\text{O}\). Precipitation with \(\text{CO}_2\) gave material \((0.58 \text{ g, 78\%})\), which was recrystallized \((\text{MeOH})\) to give \(3\text{h}\) \((0.47 \text{ g, 63\%})\): mp 273-273.5 °C; UV \((\text{MeOH})\) max 289 nm \((\varepsilon 13\ 400)\), min 240 nm \((\varepsilon 3900)\); \(^1\text{H} \) NMR \((500 \text{ MHz, DMSO-}d_6\) \(\delta 14.04 \text{ (s, 1 H), 8.69 \text{ (s, 1 H), 8.49 \text{ (s, 1 H), 7.10-7.23 \text{ (m, 6 H), 4.61 \text{ (s, 2 H)\)}}\)); \(^{13}\text{C} \) NMR \((125 \text{ MHz, DMSO-}d_6\) \(\delta 156.1, 150.9, 147.7, 145.9, 137.6, 128.5, 128.0, 126.1, 121.7, 120.9, 35.4; \text{HRMS m/z} 333.0634 (\text{MNa}^+ [\text{C}_{15}\text{H}_{11}\text{ClN}_6\text{Na}]) = \)
333.0631). Anal. Calcd for C_{15}H_{11}ClN_{6}: C, 57.98; H, 3.57; N, 27.05. Found: C, 58.03; H, 3.70; N, 27.18.

**2-Chloro-6-(4,5-diphenylimidazol-1-yl)purine (3i)**

9-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-2-chloro-6-(4,5-diphenylimidazol-1-yl)purine (11i) (1.41 g, 1.7 mmol) was dissolved in HOAc (69 mL). To the solution was added AcCl (0.68 mL, 0.75 g, 9.6 mmol), and the mixture was stirred at 65 °C for 60 h in a sealed flask (reaction complete, TLC). Volatiles were evaporated in vacuo, and the residue was washed (CH_{2}Cl_{2}) and dissolved in 0.1 N NaOH/H_{2}O. Precipitation with CO_{2} gave material (0.41 g, 67%), which was recrystallized (MeOH) to give 3i: mp 277.5-278 °C; UV (MeOH) max 277 nm (ε 16 100), min 264 nm (ε 14 800); ^1H NMR (500 MHz, DMSO-d_{6}) δ 14.04 (s, 1H), 8.84 (s, 1H), 8.73 (s, 1H), 7.20–7.49 (m, 10H); ^13C NMR (125 MHz, DMSO-d_{6}) δ 157.2, 151.6, 147.4, 146.2, 139.7, 139.5, 134.3, 131.3, 131.1, 129.02, 128.98, 128.92, 128.08, 127.8, 127.6, 124.0; HRMS m/z 395.0792 (MNa^+) [C_{20}H_{13}ClN_{6}Na] = 395.0788). Anal. Calcd for C_{20}H_{13}ClN_{6}: C, 64.43; H, 3.51; N, 22.54. Found: C, 64.29; H, 3.78; N, 22.53.

**9-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-2-chloro-6-(2-propylimidazol-1-yl)purine (6)**

Method 1. A mixture of 2’,3’,5’-tri-O-acetyl-2-N-tritylguanosine (5.92 g, 9.1 mmol), I_{2} (11.55 g, 45.5 mmol), Ph_{3}P (11.93 g, 45.5 mmol) and 2a (5.01 g, 45.5 mmol) was stirred in toluene (180 mL) at 95 °C for 15 min. DIPEA (15.9 mL, 11.80 g, 91.3 mmol) was added, and the mixture was stirred at 95 °C overnight. After removal of volatiles in vacuo, the residue was extracted with boiling EtOAc. The combined EtOAc extracts were evaporated to dryness, and the residue was dried under vacuum. The material obtained
was stirred in TFA/H₂O (9:1, 250 mL) at 0 °C for 4 h. Volatiles were evaporated in vacuo, and the residue was chromatographed (CH₂Cl₂ → MeOH/CH₂Cl₂, 1:12). This solid material was treated with charcoal in MeOH. Volatiles were evaporated in vacuo, and the residue was dissolved in CH₂Cl₂ and washed (NaHCO₃/H₂O, brine) and dried (Na₂SO₄) to give 5 as a colored solid (3.20 g, 81%, contaminated with Ph₃PO).

To a stirred solution of 5 (2.37 g, 4.72 mmol) in CH₂Cl₂ (120 mL) was added TMSCl (5.3 mL, 4.54 g, 42.5 mmol) dropwise under N₂, and then BTEANO₂ (7.1 g, 29.8 mmol) in CH₂Cl₂ (40 mL). Evolution of gas was observed, and when this subsided, additional TMSCl (5.3 mL) was added. The mixture was then stirred at ambient temperature for 3 h. The solution was diluted with CH₂Cl₂ and washed (NaHCO₃/H₂O, 2 x 200 mL + 100 mL), and the aqueous layer was extracted with CH₂Cl₂. The combined organic phase was dried (Na₂SO₄), and volatiles were evaporated in vacuo. The residue was chromatographed (MeOH/CH₂Cl₂, 1:99-1:90) to give crude product (1.40 g, 57%, contaminated with Ph₃PO), which was recrystallized (i-PrOH) to give 6: mp 126-127.5 °C; UV (MeOH) max 217, 287 nm (ε 25 300, 15 000), min 238, 261 nm (ε 4200, 6200);

¹H NMR (500 MHz, CDCl₃) δ 8.57 (d, J = 1.8 Hz, 1H), 8.25 (s, 1H), 7.11 (d, J = 1.5 Hz, 1H), 6.27 (d, J = 5.5 Hz, 1H), 5.83 (t, J = 5.5 Hz, 1H), 5.60–5.62 (m, 1H), 4.49–4.51 (m, 1H), 4.43–4.44 (m, 2H), 3.29 (t, J = 7.7 Hz, 2H), 2.19 (s, 3H), 2.17 (s, 3H), 2.11 (s, 3H), 1.86 (sext, J = 7.6 Hz, 2H), 1.07 (t, J = 7.7 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 170.5, 169.9, 169.7, 154.6, 153.8, 151.6, 148.4, 142.5, 129.3, 123.0, 120.5, 86.6, 81.0, 73.5, 70.8, 63.2, 33.2, 21.6, 21.1, 20.8, 20.7, 14.2; HRMS m/z 520.1476 (M⁺ [C₂₂H₂₃ClN₆O₇] = 520.1473). Anal. Calcd For C₂₂H₂₃ClN₆O₇: C, 50.73; H, 4.84; N, 16.13. Found: C, 50.58; H, 4.87; N, 16.15.
Method 2. A mixture of 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-2,6-dichloropurine (10) (1.12 g, 2.5 mmol) and 2a (2.20 g, 20 mmol) was dissolved in CH$_3$CN (30 mL) and stirred at 65 °C under N$_2$ for 2 h (reaction complete, TLC). After removal of volatiles, the residue was dissolved in CH$_2$Cl$_2$ (200 mL) and washed (H$_2$O, 3 x 50 mL). The aqueous phase was extracted with CH$_2$Cl$_2$, and the combined organic phase was dried (Na$_2$SO$_4$) and evaporated to dryness. The residue was chromatographed (MeOH/CH$_2$Cl$_2$, 1:95) to give 6 (977 mg, 93%).

An extended reaction time (20 h) caused minor formation of bis-substituted product: LRMS m/z 594 (M$^+\left[C_{28}H_{34}N_8O_7\right] = 594)$.

9-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-2-chloro-6-(2-isopropylimidazo-1-yl)purine (11d)

A solution of 10 (2.12 g, 4.76 mmol) and 2d (5.23 g, 47.6 mmol) in CH$_3$CN (60 mL) was stirred at 65 °C under N$_2$ overnight (reaction complete, TLC). Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH$_2$Cl$_2$, 1:90) to give 11d as a solid (quantitative, contaminated with 2d): $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.47 (s, 1H), 8.24 (s, 1H), 7.10 (s, 1H), 6.23 (d, $J = 5.4$ Hz, 1H), 5.82 (t, $J = 5.5$ Hz, 1H), 5.60 (t, $J = 5.5$ Hz, 1H), 4.42–4.49 (m, 3H), 4.07 (sept, $J = 6.8$ Hz, 1H), 2.17 (s, 3H), 2.16 (s, 3H), 2.10 (s, 3H), 1.41 (d, $J = 7.0$ Hz, 3H), 1.40 (d, $J = 7.0$ Hz, 3H); HRMS m/z 521.1561 (MH$^+\left[C_{22}H_{26}ClN_6O_7\right] = 521.1552$).

9-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-6-(2-butylimidazol-1-yl)-2-chloropurine (11e)

A solution of 10 (2.41 g, 5.4 mmol) and 2e (6.68 g, 54 mmol) in CH$_3$CN (60 mL) was stirred at 65 °C under N$_2$ for 32 h (reaction complete, TLC). Volatiles were
evaporated in vacuo, and the residue was chromatographed (MeOH/CH₂Cl₂, 1:90) to give crude 11e (3.33 g, contaminated with 2e): ¹H NMR (500 MHz, CDCl₃) δ 8.56 (s, 1H), 8.24 (s, 1H), 7.10 (s, 1H), 6.26 (d, J = 5.8 Hz, 1H), 5.83 (t, J = 5.6 Hz, 1H), 5.61 (t, J = 5.6 Hz, 1H), 4.43–4.51 (m, 3H), 3.31 (t, J = 7.9 Hz, 2H), 2.18 (s, 3H), 2.16 (s, 3H), 2.11 (s, 3H), 1.81 (quint, J = 7.7 Hz, 2H), 1.50 (sext, J = 7.7 Hz, 2H), 0.98 (t, J = 7.3 Hz, 3H); HRMS m/z 535.1702 (MH⁺ [C₂₃H₂₈ClN₆O₇] = 535.1708).

9-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-2-chloro-6-(2-pentylimidazol-1-yl)purine (11f)

A solution of 10 (1.1 g, 23.7 mmol) and 2f (3.27 g, 23.7 mmol) in CH₃CN (25 mL) was stirred at 65 °C under N₂ overnight (reaction complete, TLC). Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH₂Cl₂, 1:90) to give crude 11f (quantitative, contaminated with traces of 2f): ¹H NMR (500 MHz, CDCl₃) δ 8.57 (s, 1H), 8.24 (s, 1H), 7.10 (s, 1H), 6.26 (d, J = 5.5 Hz, 1H), 5.83 (t, J = 5.5 Hz, 1H), 5.60–5.61 (m, 1H), 4.44–4.50 (m, 3H), 3.31 (t, J = 7.5 Hz), 2.20 (s, 3H), 2.17 (s, 3H), 2.11 (s, 3H), 1.83 (quint, J = 7.5 Hz, 2H), 1.38–1.49 (m, 4H), 0.93 (t, J = 7.3 Hz, 3H); HRMS m/z 549.1858 (MH⁺ [C₂₄H₃₀ClN₆O₇] = 549.1865).

9-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-2-chloro-6-[2-(2-phenylpropyl)imidazol-1-yl]purine (11g)

A solution of 10 (0.65 g, 1.45 mmol) and 2g (2.69 g, 14.5 mmol) in CH₃CN (20 mL) was stirred at 65 °C under N₂ for 17 h (reaction complete, TLC). Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH₂Cl₂, 1:90 → 1:30) to give a diastereomeric mixture (0.73 g, 84%). Recrystallization (i-PrOH) gave diastereomeric 11g (0.59 g, 68%): mp 160-161.5 °C; UV (MeOH) max 258, 288 nm (ε 12
900, 5600), min 239, 262 nm (ε 4100, 5600); \(^1\)H NMR (500 MHz, CDCl\(_3\) \(\delta\) 8.33 (8.31) (d, \(J = 1.5\) Hz, 1H), 8.20 (8.19) (s, 1H), 7.04–7.11 (m, 5H), 6.88–6.93 (m, 1H), 6.24 (6.23) (d, \(J = 5.5\) Hz, 1H), 5.82–5.85 (m, 1H), 5.61–5.63 (m, 1H), 4.41–4.51 (m, 3H), 3.75–3.83 (m, 1H), 3.53–3.60 (m, 1H), 3.31–3.37 (m, 1H), 2.13–2.22 (m, 9H), 1.34 (d, \(J = 7.0\) Hz, 3H); \(^13\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 170.4, 169.8, 169.6, 154.5, 153.6, 149.7 (149.6), 148.4, 146.1 (146.0), 142.5, 129.4, 128.2, 127.2, 125.9, 123.2, 120.6, 86.6, 81.0, 73.5, 70.9, 63.2, 39.9 (39.7), 38.7, 21.1 (21.0), 20.8 , 20.6 ; HRMS \(m/z\) 619.1685 (MNa\(^+\) [C\(_{28}\)H\(_{29}\)ClN\(_6\)O\(_7\)Na] = 619.1684). Anal. Calcd for C\(_{28}\)H\(_{29}\)ClN\(_6\)O\(_7\): C, 56.33; H, 4.90; N, 14.08. Found: C, 56.18; H, 5.00; N, 14.06.

9-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-6-(2-benzylimidazol-1-yl)-2-chloropurine (11h)

A solution of 10 (0.734 g, 1.64 mmol) and 2h (2.61 g, 16.5 mmol) in CH\(_3\)CN (30 mL) was stirred at 65 °C under N\(_2\). The reaction was complete in 12 h, and volatiles were evaporated in vacuo. The residue was chromatographed twice (EtOAc/hexanes, 1:1 → 7:3; MeOH/CH\(_2\)Cl\(_2\), 1:90) to give 11h (0.77 g, 79%): mp 154.5-156 °C; UV (MeOH) max 288 nm (ε 14 600), min 240 nm (ε 4100); \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 8.68 (d, \(J = 1.9\) Hz, 1H), 8.21 (s, 1H), 7.33 (d, \(J = 7.0\) Hz, 2H), 7.23 (t, \(J = 7.4\) Hz, 2H), 7.17 (d, \(J = 1.9\) Hz, 1H), 7.15 (t, \(J = 7.3\) Hz, 1H), 6.11 (d, \(J = 5.5\) Hz, 1H), 5.78 (“t”, \(J = 5.6\) Hz, 1H), 5.59 (dd, \(J = 5.5, 6.7\) Hz, 1H), 4.78 (s, 2H), 4.40–4.48 (m, 3H), 2.17 (s, 3H), 2.14 (s, 3H), 2.09 (s, 3H); \(^13\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 170.2, 169.6, 169.4, 154.3, 153.4, 149.2, 147.7, 142.3, 137.4, 129.4, 129.1, 128.2, 126.3, 122.5, 120.9, 86.2, 80.7, 73.2, 70.6, 62.9, 36.9, 20.8, 20.6, 20.4; HRMS \(m/z\) 591.1355 (MNa\(^+\) [C\(_{28}\)H\(_{29}\)ClN\(_6\)O\(_7\)Na] = 591.1371).
Anal. Calcd for C_{26}H_{25}ClN_{6}O_{7}: C, 54.89; H, 4.43; N, 14.77. Found: C, 54.67; H, 4.60; N, 14.58.

9-(2,3,5-Tri-O-acetyl-α-D-ribofuranosyl)-2-Chloro-6-(4,5-diphenylimidazol-1-yl)purine (11i)

A solution of 10 (0.45 g, 1.0 mmol) and 4,5-diphenylimidazole (2.21 g, 10 mmol) in DMF (15 mL) was stirred at 65 °C under N_{2} for 67 h (reaction almost complete, TLC). Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH_{2}Cl_{2}, 1:90) to give 11i (0.53 g, 83%) and a mixture of 4,5-diphenylimidazole (19 mg) and 11i (52 mg, 91% total). Recrystallization (i-PrOH) gave 11i: mp 146-146.5 °C; UV (MeOH) max 279 nm (ε 18 300), min 267 nm (ε 16 800); \(^1\)H NMR (500 MHz, CDCl_{3}) δ 9.03 (s, 1H), 8.22 (s, 1H), 7.55–7.57 (m, 2H), 7.35–7.40 (m, 4H), 7.21–7.27 (m, 4H), 6.21 (d, \(J=5.5\) Hz, 1H), 5.80 (‘t’, \(J=5.6\) Hz, 1H), 5.58 (‘t’, \(J=5.1\) Hz, 1H), 4.41–4.48 (m, 3H), 2.16 (s, 3H), 2.15 (s, 3H), 2.09 (s, 3H); \(^{13}\)C NMR (125 MHz, CDCl_{3}) δ 170.2, 169.5, 169.4, 154.3, 153.5, 147.4, 142.9, 140.5, 139.2, 133.5, 131.1, 131.0, 128.33, 128.28, 128.17, 127.5, 127.2, 124.1, 86.4, 80.7, 73.2, 70.5, 62.9, 20.8, 20.5, 20.4; HRMS m/z 631.1694 (MNa\(^{+}\) [C_{31}H_{28}ClN_{6}O_{7}Na] = 631.1708). Anal. Calcd for C_{31}H_{28}ClN_{6}O_{7}: C, 59.00; H, 4.31; N, 13.32. Found: C, 58.89; H, 4.45; N, 13.24.

General method 1 for 12 and 13. A mixture of the 6-(heteroaryl)purine (1 mmol) and sodium hydride (0.06 g, 60% w/w suspension, 1.5 mmol) in a dried polar solvent (A) was stirred at ambient temperature under N_{2} for 2 h. A solution of 2-deoxy-3,5-dio-\((p\)-toluoyl)-α-D-erythro-pentofuranosyl chloride (1.8 mmol) in a less polar dried solvent (B) was added with a syringe. The mixture was stirred for 22 h, and volatiles were evaporated in vacuo.
General method 2 for 12 and 13. A mixture of 6-(2-alkylimidazol-1-yl)-2-chloropurine (1 mmol) and sodium hydride (60% w/w suspension, 1.5 mmol) in dried CH$_3$CN (10 mL) was stirred at ambient temperature under N$_2$ for 8 h. The solution was chilled to 0 °C, and a solution of 2-deoxy-3,5-di-O-(p-toluoyl)-α-D-erythro-pentofuranosyl chloride (1.8 mmol) in cold, dried CH$_2$Cl$_2$ (10 mL, 0 °C) was added with a syringe. The reaction mixture was then stirred for 22 h, and allowed to gradually warm to ambient temperature. Volatiles were evaporated in vacuo and the residue was chromatographed (25 g silica gel, MeOH/CH$_2$Cl$_2$, 1:30).

General method 3 for 16 and 18. The 6-(2-alkylimidazol-1-yl)-2-chloro-9-[2-deoxy-3,5-di-O-(p-toluoyl)-β-D-erythro-pentofuranosyl]purine (1 mmol) was added to 0.3 M BnI/CH$_3$CN (40 mL, 12 mmol), which was prepared in situ from NaI (15 g, 94 mmol) and BnCl (3.5 mL, 3.85 g, 30.4 mmol) in CH$_3$CN (100 mL). The mixture was stirred at 60 °C for 1.5 h. Removal of volatiles and chromatography (MeOH/CH$_2$Cl$_2$, 1:90 → 1:30) gave the benzylimidazolium iodide salt as yellow foam, which was transferred into a pressure flask and cooled at -4 °C. Cold NH$_3$/MeOH (26%, 50 mL) was added, and the sealed mixture was stirred at 60 °C for 11 h. Volatiles were evaporated, and the residue was chromatographed [Dowex 1 x 2 (OH$^-$) resin, H$_2$O/MeOH, 1:0 → 3:2] to give 2-chloro-2’-deoxyadenosine.

2-Chloro-9-[2-deoxy-3,5-di-O-(p-toluoyl)-β-D-erythro-pentofuranosyl]-6-(2-propylimidazol-1-yl)purine (12a)

The sodium salt of 3a (0.13 g, 0.5 mmol) in dried CH$_3$CN (10 mL) was treated with 2-deoxy-3,5-di-O-(p-toluoyl)-α-D-erythro-pentofuranosyl chloride (0.30 g, 0.8
<table>
<thead>
<tr>
<th>A</th>
<th>#</th>
<th>A + B</th>
<th>α/β</th>
<th>%*</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMF (5 mL)</td>
<td>1</td>
<td>DMF (10 mL)/toluene (5 mL)</td>
<td>1.0:1.0</td>
<td>85</td>
</tr>
<tr>
<td>CH₃CN (5 mL)</td>
<td>2</td>
<td>CH₃CN (5 mL)/toluene (5 mL)</td>
<td>1.0:3.6**</td>
<td>85</td>
</tr>
<tr>
<td>CH₃CN (5 mL)</td>
<td>3</td>
<td>CH₃CN (5 mL)/toluene (5 mL)</td>
<td>0.0:100+</td>
<td>80</td>
</tr>
<tr>
<td>CH₃CN (5 mL)</td>
<td>4</td>
<td>CH₃CN (5 mL)/toluene (5 mL)</td>
<td>1.0:2.4**</td>
<td>93</td>
</tr>
<tr>
<td>CH₃CN (10 mL)</td>
<td>5</td>
<td>CH₃CN (10 mL)/toluene (10 mL)</td>
<td>1.0:22.0</td>
<td>&gt;100</td>
</tr>
<tr>
<td>CH₃CN (30 mL)</td>
<td>6</td>
<td>CH₃CN (30 mL)/toluene (30 mL)</td>
<td>1.0:6.7</td>
<td>82</td>
</tr>
<tr>
<td>CH₃CN (30 mL)</td>
<td>7</td>
<td>CH₃CN (30 mL)/toluene (40 mL)</td>
<td>1.0:10.3 (1.0:7.9)#</td>
<td>77</td>
</tr>
<tr>
<td>CH₃CN (5 mL)</td>
<td>8</td>
<td>CH₃CN (5 mL)/toluene (5 mL)</td>
<td>0.9:99.1</td>
<td>&gt;100</td>
</tr>
<tr>
<td>CH₃CN (5 mL)</td>
<td>9</td>
<td>CH₃CN (5 mL)/toluene (5 mL)</td>
<td>0.4:99.6</td>
<td>&gt;100</td>
</tr>
<tr>
<td>CH₃CN (10 mL)</td>
<td>10</td>
<td>CH₃CN (10 mL)/CH₂Cl₂ (10 mL), 0 °C</td>
<td>0.0:100</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

* Chromatography (MeOH/CH₂Cl₂, 1:30); ** Sugar chloride may not have been good in spite of its verification by ¹H NMR; +300 MHz ¹H NMR; +Values in () were measured before chromatography.

**Table 1. Stereoselectivity for glycosylation of the sodium salt of 2-chloro-6-(2-propylimidazol-1-yl)purine**

mmol) in CH₂Cl₂ (10 mL) by general method 2. No α-nucleoside was detected by ¹H NMR. Column chromatography was performed twice (25 g silica gel, MeOH/CH₂Cl₂, 1:30, and EtOAc/hexanes, 1:1) to give the β-anomer 12a (0.26 g, 83%). Recrystallization (EtOAc) gave analytically pure 12a (0.17 g, 55%): mp 192-193°C.; UV (MeOH) max 220, 239, 287 nm (ε 40 700, 38 300, 16 700), min 231, 265 nm (ε 35 900, 10 300); ¹H NMR (500 MHz, CDCl₃) δ 8.52 (s, 1H), 8.27 (s, 1H), 8.00 (d, J = 7.8 Hz, 2H), 7.86 (d, J
= 7.8 Hz, 2H), 7.32 (d, J = 7.8 Hz, 2H), 7.28 (d, J = 7.8 Hz, 2H), 7.20 (s, 1H), 6.61 (t, J = 7.1 Hz, 1H), 5.82–5.83 (m, 1H), 4.84–4.68 (m, 3H), 3.29 (t, J = 7.8 Hz, 2H), 2.98–3.01 (m, 2H), 2.47 (s, 3H), 2.38 (s, 3H), 1.86 (sext, J = 7.5 Hz, 2H), 1.07 (t, J = 7.3 Hz, 3H); NOE difference: H1’ was irradiated, and enhancement of H4’ (small), H8 and H2’,2” signals was observed; 13C NMR (125 MHz, CDCl3) δ 166.29, 166.23, 154.38, 153.42, 151.47, 148.18, 144.97, 144.65, 142.40, 130.12, 129.78, 129.61, 129.56, 129.20, 126.63, 126.44, 122.95, 120.57, 85.46, 83.82, 75.19, 64.12, 38.88, 33.12, 22.03, 21.91, 21.60, 14.25; HRMS m/z 637.1940 (MNa+ [C32H31ClN6O5Na = 637.1942]). Anal. Calcd for C32H31ClN6O5: C, 62.49; H, 5.08; N, 13.66. Found: C, 62.44; H, 5.18; N, 13.72.

This reaction was repeated on a larger scale with the sodium salt of 3a (1.54 g, 5.87 mmol) in dried CH3CN (100 mL) treated with 2-deoxy-3,5-di-O-(p-toloyl)-α-D-erythro-pentofuranosyl chloride (3.74 g, 9.62 mmol) in CH2Cl2 (100 mL) by general method 2 for 5 h (reaction complete, TLC). Sampling at different reaction times showed no α-nucleoside by 1H NMR (500 MHz). Volatiles were evaporated, and the residue was dissolved in CH2Cl2. The solution was washed (H2O) and dried (Na2SO4), and volatiles were evaporated in vacuo. The residue was chromatographed (EtOAc/hexanes, 1:1 → 7:3) to give 12a (3.42 g, 95%). Recrystallization from EtOAc gave the β-anomer (2.75 g, 76%).

2-Chloro-9-[2-deoxy-3,5-di-O-(p-toloyl)-β-D-erythro-pentofuranosyl]-6-(2-isopropylimidazol-1-yl)purine (12d)

The sodium salt of 3d (0.132 g, 0.5 mmol) in dried CH3CN (10 mL) was treated with 2-deoxy-3,5-di-O-(p-toloyl)-α-D-erythro-pentofuranosyl chloride (0.334 g, 0.86
mmol) in CH₂Cl₂ (10 mL) by general method 2 for 1 h (reaction complete, TLC).

Sampling of the reaction mixture at the end of the reaction time showed no α-nucleoside by ¹H NMR (500 MHz). The residue was chromatographed (25g silica gel, EtOAc/hexanes~1:1) to give 12d (quantitative). Recrystallization (EtOAc) gave 12d (0.22 g, 70%): UV (MeOH) max 223, 241, 285 nm (ε 32 100, 35 400, 14 800), min 230, 265 nm (ε 30 300, 9200); ¹H NMR (500 MHz, CDCl₃) δ 8.42 (d, J = 1.0 Hz, 1H), 8.26 (s, 1H), 7.99 (d, J = 7.8 Hz, 2H), 7.85 (d, J = 8.3 Hz, 2H), 7.30 (d, J = 7.8 Hz, 2H), 7.20 (d, J = 8.8 Hz, 2H), 7.11 (d, J = 1.0 Hz, 1H), 6.60 (t, J = 6.8 Hz, 1H), 5.81 (br s, 1H), 4.78–4.83 (m, 1H), 4.67–4.71 (m, 2H), 4.08 (sept, J = 6.8 Hz, 1H), 2.97–3.01 (m, 2H), 2.46 (s, 3H), 2.37 (s, 3H), 1.43 (d, J = 6.8 Hz, 3H), 1.41 (d, J = 6.8 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 166.05, 165.99, 156.18, 154.20, 153.21, 148.15, 144.72, 144.40, 142.25, 129.88, 129.55, 129.36, 129.33, 128.82, 126.44, 126.26, 123.00, 120.38, 85.23, 83.59, 74.93, 63.87, 38.63, 28.81, 21.78, 21.61; HRMS m/z 637.1931 (MNa⁺ [C₃₂H₃₁Cl₅N₆O₅Na = 637.1942]).

This reaction was repeated on a larger scale with the sodium salt of 3d (902 mg, 3.43 mmol) in dried CH₃CN (70 mL) treated with 2-deoxy-3,5-di-O-(p-toluoyl)-α-D-erythro-pentofuranosyl chloride (2.62 g, 6.73 mmol) in CH₂Cl₂ (70 mL) by general method 2 for 5 h. Sampling of the reaction mixture showed traces of α-nucleoside by ¹H NMR (500 MHz) (< 1:20). Column chromatography (EtOAc/hexanes, 1:1 → 7:3) gave the β-anomer (quantitative, with traces of α-nucleoside). Recrystallization (EtOAc) gave the β-anomer 12d (1.76 g, 84%).
The reaction was performed in DMF with 3d (0.13 g, 0.5 mmol) by method 1. The residue was chromatographed (EtOAc/hexanes, 1:1) to give the crude α-nucleoside (0.108 g) and an α/β-nucleoside mixture (0.218 g, α/β = 0.76). α-nucleoside: ¹H NMR (500 MHz, CDCl₃) δ 8.48 (s, 1H), 8.41 (s, 1H), 7.96 (d, J = 8.3 Hz, 2H), 7.57 (d, J = 8.2 Hz, 2H), 7.29 (d, J = 8.5 Hz, 2H), 7.15 (s, 1H), 7.13 (d, J = 8.0 Hz, 2H), 6.66 (d, J = 6.1 Hz, 1H), 5.72 (br s, 1H), 4.96 (br s, 1H), 4.62–4.67 (m, 2H), 4.13 (sept, J = 7.0 Hz, 1H), 3.06–3.13 (m, 2H), 2.45 (s, 3H), 2.35 (s, 3H), 1.40–1.41 (m, 6H).

6-(2-Butylimidazol-1-yl)-2-chloro-9-[2-deoxy-3,5-di-O-(p-toluoyl)-β-D-erythro-pentofuranosyl]purine (12e)

The sodium salt of 3e (0.139 g, 0.5 mmol) in dried CH₃CN (10 mL) was treated with 2-deoxy-3,5-di-O-(p-toluoyl)-α-D-erythro-pentofuranosyl chloride (0.334 g, 0.86 mmol) in CH₂Cl₂ (10 mL) by general method 2. Sampling of the reaction mixture showed traces of α-nucleoside by ¹H NMR (500 MHz) (1:24). Volatiles were evaporated in vacuo, and the residue was chromatographed (25g silica gel, EtOAc/hexanes, 3:7 → EtOAc) to give the β-anomer (274 mg, 86%) with traces of the α-anomer. Recrystallization (EtOAc/hexanes) gave the β-anomer 12e: UV (MeOH) max 223, 241, 287 nm (ε 29 900, 33 400, 13 200), min 230, 265 nm (ε 28 100, 7500); ¹H NMR (500 MHz, CDCl₃) δ 8.51 (s, 1H), 8.26 (s, 1H), 7.98 (d, J = 8.0 Hz, 2H), 7.84 (d, J = 8.0 Hz, 2H), 7.30 (d, J = 8.0 Hz, 2H), 7.19 (d, J = 8.0 Hz, 2H), 7.09 (s, 1H), 6.60 (t, J = 6.9 Hz, 1H), 5.80 (br s, 1H), 4.78–4.81 (m, 1H), 4.66–4.70 (m, 2H), 3.31 (t, J = 7.8 Hz, 2H), 2.97–3.00 (m, 2H), 2.46 (s, 3H), 2.37 (s, 3H), 1.80 (quint, J = 7.4 Hz, 2H), 1.50 (sext, J = 7.4 Hz, 2H), 0.97 (t, J = 7.5 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 166.05, 165.98,
α-Anomer: $^1$H NMR (500 MHz, CDCl$_3$) δ 8.59 (s, 1H), 8.41 (s, 1H), 7.97 (d, $J$ = 8.3 Hz, 2H), 7.57 (d, $J$ = 8.3 Hz, 2H), 7.30 (d, $J$ = 8.0 Hz, 2H), 7.13–7.14 (m, 3H), 6.67 (dd, $J$ = 1.8, 6.4 Hz, 1H), 5.72–5.73 (m, 1H), 4.96–4.97 (m, 2H), 4.62–4.70 (m, 2H), 3.31 (t, $J$ = 7.8 Hz, 2H), 3.06–3.15 (m, 2H), 2.46 (s, 3H), 2.14 (s, 3H), 1.80 (quint, $J$ = 7.4 Hz, 2H), 1.50 (sext, $J$ = 7.4 Hz, 2H), 0.97 (t, $J$ = 7.3 Hz, 3H).

2-Chloro-9-[2-deoxy-3,5-di-O-(p-toluoyl)-β-D-erythro-pentofuranosyl]-6-(2-pentylimidazol-1-yl)purine (12f)

The sodium salt of 3f (0.147 g, 0.5 mmol) in dried CH$_3$CN (10 mL) was treated with 2-deoxy-3,5-di-O-(p-toluoyl)-α-D-erythro-pentofuranosyl chloride (0.334 g, 0.86 mmol) in CH$_2$Cl$_2$ (10 mL) by general method 2. Sampling of the reaction mixture showed traces of α-nucleoside by $^1$H NMR (500 MHz) (1:62). Volatiles were evaporated in vacuo, and the residue was chromatographed (25g silica gel, EtOAc/hexanes, 1:1) to give 12f (quantitative, with traces of α-anomer). Recrystallization (EtOAc/hexanes) gave the β-anomer 12f: UV (MeOH) max 223, 241, 287 nm ($\epsilon$ 32 100, 34 700, 15 000), min 231, 265 nm (ε 30 000, 9300); $^1$H NMR (500 MHz, CDCl$_3$) δ 8.50 (s, 1H), 8.26 (s, 1H), 7.98 (d, $J$ = 8.2 Hz, 2H), 7.84 (d, $J$ = 7.9 Hz, 2H), 7.30 (d, $J$ = 7.9 Hz, 2H), 7.19 (d, $J$ = 7.9 Hz, 2H), 7.09 (s, 1H), 6.60 (t, $J$ = 7.0 Hz, 1H), 5.80 (br s, 1H), 4.80–4.81 (m, 1H), 4.67–4.69 (m, 2H), 3.28–3.29 (m, 2H), 2.98–3.03 (m, 2H), 2.46 (s, 3H), 2.37 (s, 3H), 1.82 (quint, $J$ = 7.6 Hz, 2H), 1.36–1.48 (m, 4H), 0.93 (t, $J$ = 7.6 Hz, 3H); $^{13}$C NMR (125
MHz, CDCl$_3$) $\delta$ 166.04, 165.99, 154.13, 153.19, 151.46, 147.95, 144.72, 144.40, 142.15, 129.87, 129.53, 129.36, 129.32, 128.94, 126.39, 126.20, 122.70, 120.32, 85.20, 83.58, 74.95, 63.87, 38.63, 31.76, 31.00, 27.71, 22.50, 21.79, 21.67, 14.10; HRMS $m/z$ 643.2426 (MH$^+$ [C$_{34}$H$_{36}$ClN$_6$O$_5$ = 643.2436]).

2-Chloro-9-[2-deoxy-3,5-di-O-(p-toluoyl)-β-D-erythro-pentofuranosyl]-6-[2-(2-phenylpropyl)imidazol-1-yl]purine (12g)

The sodium salt of 3g (0.17 g, 0.5 mmol) in dried CH$_3$CN (10 mL) was treated with 2-deoxy-3,5-di-O-(p-toluoyl)-α-D-erythro-pentofuranosyl chloride (0.334 g, 0.86 mmol) in CH$_2$Cl$_2$ (10 mL) by general method 2. Sampling of the reaction mixture showed a minimally detectable amount of α-nucleoside by $^1$H NMR (500 MHz). Volatiles were evaporated in vacuo, and the residue was chromatographed (25g silica gel, EtOAc/hexanes, 1:1) to give the diastereomeric β-anomer (0.343 g, 99%) with a trace of α-nucleoside. Recrystallization (EtOAc/hexanes) gave the 12g diastereomers: UV (MeOH) max 241, 285 nm ($\epsilon$ 33 300, 11 000), min 222, 266 nm ($\epsilon$ 27 200, 6900); $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.30 (8.28) (s, 1H), 8.22 (s, 1H), 7.98–8.00 (m, 2H), 7.85–7.89 (m, 2H), 7.18–7.31 (m, 4H), 7.06–7.12 (m, 5H), 6.90 (br s, 1H), 6.58–6.59 (m, 1H), 5.80 (s, 1H), 4.80–4.82 (m, 1H), 4.68–4.70 (m, 2H), 3.75–3.82 (m, 1H), 3.55–3.60 (m, 1H), 3.30–3.36 (m, 1H), 2.94–2.99 (m, 2H), 2.45 (s, 3H), 2.36 (s, 3H), 1.32 (d, $J$ = 7.0 Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 166.1, 166.0, 154.1, 153.0, 149.4, 149.3, 147.9, 145.9, 145.8, 144.7, 144.4, 142.3, 129.9, 129.6, 129.4, 128.9, 128.0, 127.01, 126.98, 126.5, 126.3, 125.7, 122.9, 120.6, 85.1, 83.6 (83.5), 74.9, 63.9, 39.6 (39.5), 38.6, 38.5, 21.8, 21.7, 20.8; HRMS $m/z$ 691.2439 (MH$^+$ [C$_{38}$H$_{36}$ClN$_6$O$_5$ = 691.2436]).
6-(2-Benzylimidazol-1-yl)-2-chloro-9-[2-deoxy-3,5-di-O-(p-toluyl)-β-D-erythro-pentofuranosyl]purine (12h)

The sodium salt of 3h (0.156 g, 0.5 mmol) in dried CH$_3$CN (10 mL) was treated with 2-deoxy-3,5-di-O-(p-toluyl)-α-D-erythro-pentofuranosyl chloride (0.334 g, 0.86 mmol) in CH$_2$Cl$_2$ (10 mL) by general method 2. Sampling of the reaction mixture showed a minimally detectable amount of α-nucleoside by $^1$H NMR (500 MHz). Volatiles were evaporated in vacuo, and the residue was chromatographed (25g silica gel, EtOAc/hexanes, 1:1) to give the β-anomer (283 mg, 85%). Recrystallization (EtOAc) gave analytically pure 12h: UV (MeOH) max 240, 289 nm (ε 35 900, 14 600), min 231, 266 nm (ε 33 500, 9300); $^1$H NMR (500 MHz, CDCl$_3$) δ 8.60 (s, 1H), 8.22 (s, 1H), 7.97 (d, $J$ = 8.3 Hz, 2H), 7.83 (d, $J$ = 9.0 Hz, 2H), 7.11–7.33 (m, 10H), 6.55 (t, $J$ = 6.8 Hz, 1H), 5.77–5.78 (m, 1H), 4.80–4.77 (m, 2H), 4.64–4.68 (m, 3H), 2.92–2.94 (m, 2H), 2.45 (s, 3H), 2.36 (s, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 166.03, 165.96, 154.01, 153.03, 149.12, 147.48, 144.71, 144.38, 142.16, 137.40, 129.85, 129.51, 129.34, 129.31, 129.23, 129.08, 128.20, 126.38, 126.29, 126.18, 122.47, 120.94, 85.13, 83.54, 74.91, 63.86, 38.60, 36.81, 21.78, 21.66; HRMS m/z 663.2108 (MH$^+$ [C$_{36}$H$_{32}$ClN$_6$O$_5$] = 663.2123).

2-Chloro-9-[2-deoxy-3,5-di-O-(p-toluyl)-β-D-erythro-pentofuranosyl]-6-(4,5-diphenylimidazol-1-yl)purine (12i)

The sodium salt of 3i (94 mg, 0.25 mmol) in dried CH$_3$CN (10 mL) was treated with 2-deoxy-3,5-di-O-(p-toluyl)-α-D-erythro-pentofuranosyl chloride (0.334 g, 0.86 mmol) in CH$_2$Cl$_2$ (10 mL) by general method 2. Sampling of the reaction mixture showed no α-nucleoside by $^1$H NMR (500 MHz). Volatiles were evaporated in vacuo, and the
residue was chromatographed (25g silica gel, EtOAc/hexanes, 3:7 → 1:1) to give the β-anomer (quantitative). Recrystallization (EtOAc/hexanes) gave 12i: UV (MeOH) max 240, 275 nm (ε 53 400, 19 300), min 223, 270 nm (ε 42 000, 19 100); 1H NMR (500 MHz, CDCl$_3$) δ 8.97 (s, 1H), 8.25 (s, 1H), 7.97 (d, $J = 7.9$ Hz, 2H), 7.86 (d, $J = 7.9$ Hz, 2H), 7.55 (d, $J = 8.2$ Hz, 2H), 7.19-7.40 (m, 13H), 6.56 (t, $J = 7.0$ Hz, 1H), 5.77–5.78 (m, 1H), 4.76–4.79 (m, 1H), 4.65–4.69 (m, 2H), 2.92–2.96 (m, 2H), 2.45 (s, 3H), 2.40 (s, 3H); 13C NMR (125 MHz, CDCl$_3$) δ 166.06, 165.94, 154.03, 153.17, 147.13, 144.71, 144.45, 142.81, 140.38, 139.24, 133.50, 131.02, 129.85, 129.55, 129.37, 129.34, 128.25, 128.17, 127.47, 127.17, 126.41, 126.18, 124.00, 85.11, 83.52, 74.85, 63.84, 38.57, 21.78, 21.71; HRMS m/z 747.2100 (MNa$^+$ [C$_{41}$H$_{33}$ClN$_6$O$_5$Na] = 747.2099).

2-Chloro-9-[2-deoxy-3,5-di-O-(p-toluoyl)-α/β-D-erythro-pentofuranosyl]-6-(imidazol-1-yl)purine (12j/13j)

<table>
<thead>
<tr>
<th>Solvent</th>
<th>#</th>
<th>Mixture</th>
<th>α/β$^+$</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMF (5 mL)</td>
<td>1</td>
<td>DMF (10 mL)/toluene (5 mL)</td>
<td>1.0:1.3</td>
<td>96</td>
</tr>
<tr>
<td>CH$_3$CN (5 mL)</td>
<td>2</td>
<td>CH$_3$CN (5 mL)/toluene (5 mL)</td>
<td>1.0:1.9</td>
<td>71</td>
</tr>
</tbody>
</table>

*Sugar chloride may not have been good in spite of its verification by 1H NMR.

**Table 2. Stereoselectivity for glycosylation of the sodium salt of 2-chloro-6-(imidazol-1-yl)purine**

The sodium salt of 2-chloro-6-(imidazol-1-yl)purine (50 mg, 0.23 mmol) in dried solvent A (10 mL) was treated with 2-deoxy-3,5-di-O-(p-toluoyl)-α-D-erythro-pentofuranosyl chloride (0.13 g, 0.35 mmol) in B (10 mL) by general method 1. The
derived residue was chromatographed to give an anomeric mixture: $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 6.66 (‘dd’, $J = 5.8$, 1.8 Hz, 1H, H$_{1',\alpha}$), 6.58 (‘t’, $J = 6.8$ Hz, 1H, H$_{1',\beta}$).

2-Amino-6-(imidazol-1-yl)-9-β-D-ribofuranosylpurine (14)

2-Acetamido-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-6-(imidazol-1-yl)purine (0.13 g, 0.25 mmol) in methanolic NH$_3$ (saturated at -10 °C) was stirred in a sealed pressure tube at ambient temperature for 15 h (reaction complete, TLC). Volatiles were evaporated in vacuo, and the residue was washed (CH$_2$Cl$_2$) to give a solid. Recrystallization (H$_2$O) gave 14 (62 mg, 72%): UV (MeOH) max 227, 321 nm ($\varepsilon$ 31 400, 9800), min 210, 281 nm ($\varepsilon$ 1400, 2300); $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 8.92 (s, 1H), 8.46 (s, 1H), 8.24 (s, 1H), 7.20 (s, 1H), 6.88 (s, 2H), 5.87 (d, $J = 5.8$ Hz, 1H), 5.50 (d, $J = 5.5$ Hz, 1H), 5.20 (d, $J = 4.0$ Hz, 1H), 5.08 (s, 1H), 4.50–4.53 (m, 1H), 4.14 (d, $J = 3.7$ Hz, 1H), 3.92–3.94 (m, 1H), 3.66 (d, $J = 11.9$ Hz, 1H), 3.56 (d, $J = 11.9$ Hz, 1H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 160.6, 156.5, 145.8, 141.7, 137.3, 130.6, 117.8, 115.8, 87.2, 86.0, 74.3, 71.0, 61.9; HRMS m/z 333.1191 (M$^+$ [C$_{13}$H$_{16}$N$_7$O$_4$] = 333.1186).

When the reaction temperature was elevated to 60 °C in a mixture of NH$_3$/H$_2$O//MeOH (28-30%, 10 mL/10 mL), a mixture of products was obtained [guanosine/2-amino-6-(imidazol-1-yl)-9-β-D-ribofuranosylpurine, 3.1:2.5].

6-Amino-2-chloro-9-β-D-ribofuranosylpurine (16)

A sample of 11a (0.89 mg, 1.71 mmol) was treated with a solution of BnI in CH$_3$CN (0.3 M, 70 mL, 21 mmol) to give 1-[9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-2-chloropurin-6-yl]-3-benzyl-2-propylimidazolium iodide: $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.97 (d, $J = 2.0$ Hz, 1H), 8.59 (s, 1H), 7.86 (d, $J = 2.5$ Hz, 1H), 7.43–7.51 (m, 5H), 6.37
(d, J = 5.5 Hz, 1H), 5.84 (t, J = 5.8 Hz, 1H), 5.80 (s, 2H), 5.58–5.60 (m, 1H), 4.45–4.54 (m, 3H), 3.70 (t, J = 7.5 Hz, 2H), 2.20 (s, 3H), 2.18 (s, 3H), 2.13 (s, 3H), 1.77 (sext, J = 7.8 Hz, 2H), 1.14 (t, J = 7.5 Hz, 3H); HRMS m/z 611.2026 (M^+ [C_{29}H_{32}ClN_6O_7] = 611.2021).

This salt was stirred in NH_3/MeOH (26%, 50 mL) at 60 °C for 11 h (reaction complete, TLC). Volatiles were evaporated in vacuo, and the residue was chromatographed (MeOH/CH_2Cl_2, 1:20 → 1:15) to give a solid (quantitative). Recrystallization (EtOH) gave 16 (92 mg). The residue derived from the mother liquor was recrystallized from H_2O to give a second crop (255 mg, 50% total): UV (MeOH) max 212, 265 nm (ε 21 200, 13 300), min 230 nm (ε 2300); ^1H NMR (500 MHz, DMSO-d_6) δ 8.38 (s, 1H), 7.86 (br s, 2H), 5.81 (d, J = 6.1 Hz, 1H), 5.48 (d, J = 6.1 Hz, 1H), 5.21 (d, J = 4.9 Hz, 1H), 5.07 (dd, J = 5.2, 6.4 Hz, 1H), 4.51 (dd, J = 6.1, 11.0 Hz, 1H), 4.10–4.13 (m, 1H), 3.92–3.94 (m, 1H), 3.64–3.68 (m, 1H), 3.53–3.57 (m, 1H); ^13C NMR (125 MHz, DMSO-d_6) δ 156.7, 152.9, 150.2, 139.9, 118.1, 87.2, 85.6, 73.5, 70.3, 61.3; HRMS m/z 301.0576 (M^+ [C_{10}H_{12}ClN_5O_4] = 301.0578).

Overnight ion exchange chromatography (Dowex 1 x 2 [OH^-], H_2O → MeOH/H_2O, 1:4 → 3:7 → 1:1 → 1:0) of the residue caused partial replacement of the 2-chloro group by methoxide to give 6-amino-2-methoxy-9-β-D-ribofuranosylpurine: UV (MeOH) max 210, 268 nm (ε 23 200, 11 300), min 228 nm (ε 2400); ^1H NMR (500 MHz, DMSO-d_6) δ 8.14 (s, 1H), 7.32 (br s, 2H), 5.79 (d, J = 6.1 Hz, 1H), 5.39 (d, J = 6.1 Hz, 1H), 5.15–5.17 (m, 2H), 4.62 (dd, J = 5.2, 6.4 Hz, 1H), 4.10–4.13 (m, 1H), 3.85–3.95 (m, 1H), 3.82 (s, 3H), 3.64–3.68 (m, 1H), 3.54–3.57 (m, 1H); LRMS m/z 297 (M^+ [C_{11}H_{13}N_3O_3] = 297).
The displacement reaction was performed at ambient temperature overnight to give a mixture of 6-amino-2-chloro-9-(β-D-ribofuranosyl)purine [UV (MeOH) max 266 nm] and 2-chloro-6-methoxy-9-(β-D-ribofuranosyl)purine [UV (MeOH) max 258 nm]. Elevation of the reaction temperature to 60 °C for 4 h gave only 6-amino-2-chloro-9-(β-D-ribofuranosyl)purine. The displaced 1-benzyl-2-propylimidazole was isolated: 1H NMR (500 MHz, DMSO-d$_6$) δ 7.29–7.40 (m, 5H), 7.20 (s, 1H), 7.18 (s, 1H), 5.23 (s, 2H), 2.57 (t, $J$ = 7.5 Hz, 2H), 1.60 (sext, $J$ = 7.3 Hz, 2H), 2.57 (t, $J$ = 7.3 Hz, 3H).

6-Amino-2-chloro-9-(2-deoxy-β-D-erythro-pentofuranosyl)purine (18)

The respective 6-(2-alkylimidazol-1-yl)-2-chloro-9-[2-deoxy-3,5-di-O-(p-toluoyl)-β-D-erythro-pentofuranosyl]purines were transformed into 2-chloro-2′-deoxyadenosine by general method 3.

1. A sample of 12a (0.615 g, 1 mmol) was treated with a solution of BnI in CH$_3$CN (0.3 M, 40 mL, 12 mmol) to give 3-benzyl-1-{2-chloro-9-[2-deoxy-3,5-di-O-(p-toluoyl)-β-D-erythro-pentofuranosyl]purin-6-yl}-2-propylimidazolium iodide (0.83 g): 1H NMR (500 MHz, CDCl$_3$) δ 8.94 (s, 1H), 8.49 (s, 1H), 8.00 (d, $J$ = 8.5 Hz, 2H), 7.88 (d, $J$ = 8.0 Hz, 2H), 7.81 (s, 1H), 7.46–7.50 (m, 5H), 7.32 (d, $J$ = 8.0 Hz, 2H), 7.25 (d, $J$ = 8.0 Hz, 2H), 6.67 (t, $J$ = 7.3 Hz, 1H), 5.75–5.85 (m, 3H), 4.71–4.82 (m, 3H), 3.67–3.74 (m, 2H), 2.99–3.02 (m, 2H), 2.47 (s, 3H), 2.42 (s, 3H), 1.75–1.81 (m, 2H), 1.17 (t, $J$ = 7.5 Hz, 3H); HRMS m/z 705.2606 (M$^+$ [C$_{39}$H$_{38}$ClN$_6$O$_5$ = 705.2592]). Deprotection [NH$_3$/MeOH (26%, 50 mL)] at 60 °C followed by ion exchange chromatography (Dowex 1 x 2 [OH$^-$/, H$_2$O/MeOH]) gave the product (quantitative). Recrystallization from EtOH gave 18 as a
white solid (0.153 g, 54%), and the residue from the mother liquor was recrystallized from H₂O to give a second crop (0.015 g, 59% total).

2. A sample of 12e (157 mg, 0.25 mmol) was treated with a solution of BnI in CH₃CN (0.48 M, 7 mL, 3.4 mmol) to give 3-benzyl-2-butyl-1-{2-chloro-9-[2-deoxy-3,5-di-O-(p-toluoyl)-β-D-erythro-pentofuranosyl]purin-6-yl}imidazolium iodide: HRMS m/z 719.2731 (M⁺ [C₄₀H₄₀ClN₆O₅ = 719.2749]). Deprotection [NH₃/MeOH (26%, 20 mL)] at 60 °C followed by ion exchange chromatography (Dowex 1 x 2 [OH⁻], H₂O/MeOH) gave 18 (61 mg, 85%).

3. A sample of 12f (150 mg, 0.24 mmol) was treated with a solution of BnI in CH₃CN (0.48 M, 6 mL, 2.9 mmol) to give 3-benzyl-1-{2-chloro-9-[2-deoxy-3,5-di-O-(p-toluoyl)-β-D-erythro-pentofuranosyl]purin-6-yl}-2-pentylimidazolium iodide: HRMS m/z 733.2892 (M⁺ [C₄₁H₄₂ClN₆O₅ = 733.2905]). Deprotection [NH₃/MeOH (26%, 30 mL)] at 60 °C followed by ion exchange chromatography (Dowex 1 x 2 [OH⁻], H₂O/MeOH) gave 18 (48 mg, 73%).

4. A sample of 12g (238 mg, 0.35 mmol) was treated with a solution of BnI in CH₃CN (0.48 M, 10 mL, 4.2 mmol) to give 3-benzyl-1-{2-chloro-9-[2-deoxy-3,5-di-O-(p-toluoyl)-β-D-erythro-pentofuranosyl]purin-6-yl}-2-(2-phenylpropyl)imidazolium iodide: HRMS m/z 781.2918 (M⁺ [C₄₅H₄₂ClN₆O₅ = 781.2905]). Deprotection [NH₃/MeOH (26%, 20 mL)] at 60 °C followed by ion exchange chromatography (Dowex 1 x 2 [OH⁻], H₂O/MeOH) gave 18 (43 mg, 42%).

2-Chloro-2'-deoxyadenosine (cladribine) (18): mp > 300 °C; UV (MeOH) max 212, 265 nm (ε 24 000, 14 600), min 229 nm (ε 2000); ¹H NMR (500 MHz, DMSO-d₆) δ
8.36 (s, 1H), 7.83 (br, 2H), 6.26 (t, J = 6.7 Hz, 1H), 5.32 (d, J = 4.3 Hz, 1H), 4.97 (t, J = 5.5 Hz, 1H), 4.38 (s, 1H), 3.85 (s, 1H), 3.57–3.61 (m, 1H), 3.48–3.53 (m, 1H), 2.62–2.67 (m, 1H), 2.25–2.29 (m, 1H); $^{13}$C NMR (125 MHz, DMSO-d$_6$) δ 157.5, 153.6, 150.8, 140.5, 118.8, 88.6, 84.2, 71.4, 62.3, 38.0; HRMS m/z 285.0615 (M$^+$ [C$_{10}$H$_{12}$ClN$_5$O$_3$] = 285.0629). Anal. Calcd for C$_{10}$H$_{12}$ClN$_5$O$_3$: C, 42.04; H, 4.23; N, 24.51. Found: C, 41.87; H, 4.50; N, 24.39.

6-Amino-2-chloropurine (19)

2,6-Dichloropurine (0.25 g, 1.31 mmol) in a pressure tube was cooled, and methanolic ammonia (27.3%, 30 mL) was added. The solution was stirred at 100 °C for 20 h, and volatiles were evaporated in vacuo. The residue was washed with CHCl$_3$ and H$_2$O to give 19 (0.19 g, 87%) with the following spectral data: $^1$H NMR (500 MHz, DMSO-d$_6$) δ 13.03 (s, 1H), 8.11 (s, 1H), 7.67 (s, 2H); $^{13}$C NMR (125 MHz, DMSO-d$_6$) δ 157.4, 153.5, 152.0, 140.2, 118.2; HRMS m/z 169.0157 (M$^+$ [C$_5$H$_4$ClN$_3$] = 169.0155).

6-Amino-2-chloro-9-[2-deoxy-3, 5-di-O-(p-toluoyl)-α/β-D-erythro-pentofuranosyl]purine (20/21)

A mixture of 19 (42 mg, 0.25 mmol) and sodium hydride (14.8 mg, 60% w/w suspension, 0.37 mmol) in dried CH$_3$CN (5 mL) was stirred at ambient temperature under N$_2$ for 9 h (turbid). 2-Deoxy-3,5-di-O-(p-toluoyl)-α-D-erythro-pentofuranosyl chloride (155 mg, 0.4 mmol) in dried toluene (5 mL) was added with a syringe, and the mixture was stirred for 12 h. Volatiles were evaporated, and the residue was chromatographed (25g silica gel, MeOH/CH$_2$Cl$_2$, 1:12) to give an anomeric mixture of 20 and 21 (quantitative, α/β~1:3.4).
This mixture was chromatographed (25g silica gel, EtOAc/hexane, 3:7 → 85:15) to give the β-anomer 20 (59 mg) and an anomeric mixture (47 mg, 82% total).

Compound 20: $^1$H NMR (500 MHz, CDCl$_3$) δ 8.00 (s, 1H), 7.98 (d, $J = 8.4$ Hz, 2H), 7.89 (d, $J = 8.4$ Hz, 2H), 7.30 (d, $J = 7.2$ Hz, 2H), 7.23 (d, $J = 8.1$ Hz, 2H), 6.53 (t, $J = 7.2$ Hz, 1H), 6.42 (br s, 2H), 5.78–5.76 (m, 1H), 4.79–4.63 (m, 3H), 2.92–2.90 (m, 2H), 2.46 (s, 3H), 2.40 (s, 3H); NOE difference: H1’ was irradiated, and enhancement of the signals for H4’ (small), H8 and H2’,2” was observed; HRMS m/z 544.1357 (MNa$^+$ [C$_{26}$H$_{24}$ClN$_5$O$_5$Na] = 544.1364).

Compound 21: $^1$H NMR (500 MHz, CDCl$_3$), δ 6.58 (“dd”, $J = 3.2$, 5.0 Hz, 1H); NOE difference: H3’ was irradiated, and enhancement of the H4’ and H2’,2’’ signals was observed; however, enhancement of the signals for H1’ was not observed. H1’ was irradiated, and enhancement of the signals for H8 and H2’,2’’ was observed, but not for that of H4’, or H3’.
5. References and Notes


32. Gupta, P. K.; Munk, S. A. Abstracts of Papers, 224th ACS National Meeting, Boston, MA, 2002; ORGN 120.


Appendix

2D NMR Spectra

1. COSY

Figure 1. 9-[2-(2-Carbamoylbenzamido)-2-deoxy-β-D-glucopyranosyl]guanine in DMSO-d$_6$/D$_2$O
Figure 2. 2-Amino-6-(imidazol-1-yl)purine in DMSO-$d_6$
Figure 3. 9-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)-6-(2,3-dihydro-2-hydroxyimidazol-1-yl)purine in CDCl₃
2. NOESY

Figure 4. 9-Cyclopentyl-6-(imidazol-1-yl)purine in CDCl₃
Figure 5. 2-Chloro-7-ethyl-6-(4,5-diphenylimidazol-1-yl)purine in DMSO-$d_6$
Figure 6. 2-Chloro-9-ethyl-6-(4,5-diphenylimidazo-1-yl)purine in DMSO-$d_6$