INVESTIGATION INTO AN IMPROVED SYNTHESIS OF 7-DEAZAGUANOSINE

By

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SYNTHESIS OF 7-DEAZAGUANOSINE

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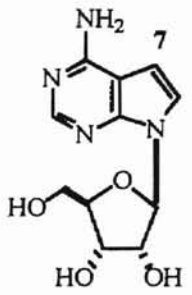
INTRODUCTION

Background and History of 7-Deazapurines

Nucleoside studies have been important to the overall understanding of the genetic code and its expression in organisms. Over 50 unusual or rare nucleosides have been detected and isolated to date, and many of these have exhibited interesting biological properties. Of these rare nucleosides, pyrrolo[2,3-*d*]pyrimidines (7-deazapurines) have received considerable attention. Pyrrolo[2,3-*d*]pyrimidines are purine analogues with a carbon atom substituted at the N-7 position of the heterocyclic base. There are two main families of 7-deazapurines: 7-deazaadenosines **1**, analogues of adenosine **2**, and 7-deazaguanosines **3**, analogues of guanosine **4**.



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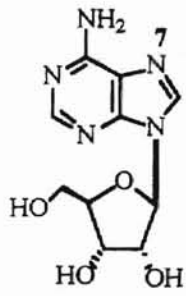

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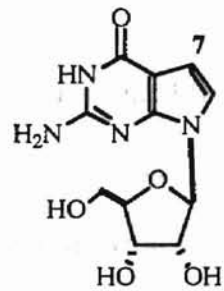

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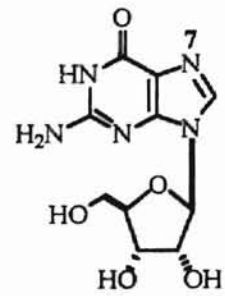
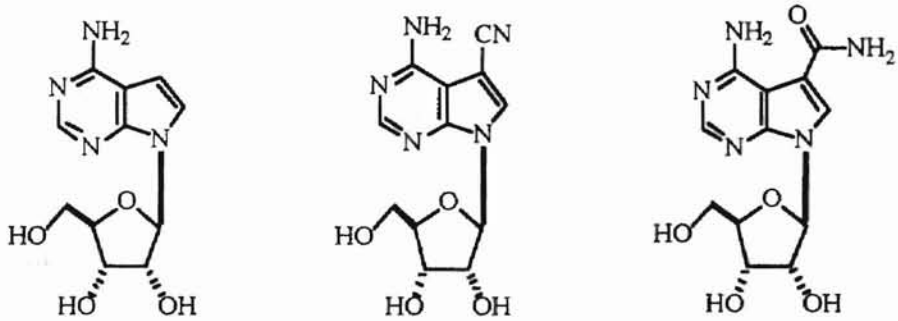
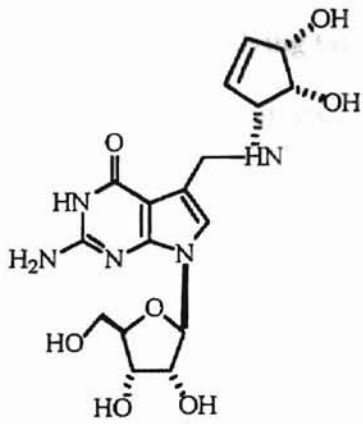


4

Several 7-deazaadenosines have been discovered and isolated from microorganisms as monomers.[1] Many of these adenosine derivatives demonstrate very active antibiotic potential, and this activity has stimulated interest in a promising new structural family of antibiotics. Tubercidin **1**, toyocamycin **5**, and sangivamycin **6** are among the most promising of this new class of nucleosides, as these three deazapurines exhibit very strong antibiotic activity.[2]



Transfer RNA (tRNA) often contains rare or unusual nitrogenous bases. Many of these interesting structures have been identified as 7-deazapurines. Several 7-deazaguanosine derivatives have been isolated from *E. coli* tRNA. Queosine **7** is an example of a 7-deazaguanosine derivative found in bacterial tRNA that may control or modulate codon/anticodon recognition.[3] An undermodified form of Queosine, 7-cyano-7-deazaguanosine **8** has been isolated from mutant *E. coli*. In other research, 7-deazapurines have exhibited antitumor and antiviral activity, making these compounds possible tools in the fight against cancer [4] and AIDS related complexes. Investigators are excited by early results with these newly encountered nucleoside analogs, making members of the 7-deazapurine family important candidates for further study.



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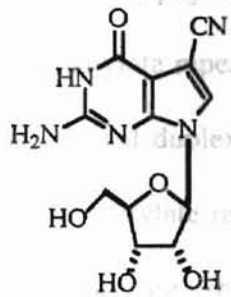

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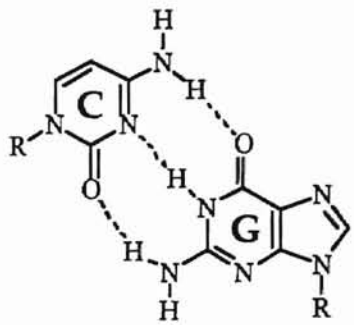
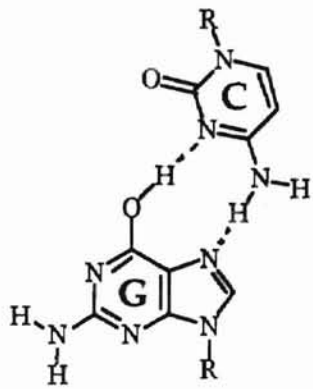
Pyrrolo[2,3-*d*]pyrimidines are capable of normal Watson-Crick base pairing which may make them useful as probes for unlocking complex DNA structures. Due to the lack of nitrogen at N-7 of the nucleoside, 7-deazapurines cannot participate in Hoogsteen base-pairing. Hoogsteen binding between two or more nucleosides is a common motif found in complex nucleoside structures such as triplex and quadraplex DNA.



Watson-Crick base pairing



Hoogsteen base pairing

Figure 1. Watson-Crick vs. Hoogsteen base-pairing between cytosine (C) and guanosine (G). Hoogsteen base pairing utilizes H-bonding at the N-7 position.

Complex DNA Structures Involving Guanosine

Deoxyguanylate readily self-associates into a gel at neutral pH and physiological salt concentration. Oligonucleotide sequences containing multiple guanylate repeats tend to form a complex DNA structure much more compact than the original duplex DNA. The electrophoretic mobility of oligonucleotides containing multiple guanylate repeating units is greatly increased when non-denaturing gels containing K^+ and Na^+ are used.[5] This self-association puzzled researchers until the crystal structure of a telomeric sequence from *Oxytricha* [6] solved the mystery of these compact associations. In this telomere, four guanosine residues from four different DNA regions are held together by Hoogsteen binding to form a square planar array. These structures are referred to as Guanosine (G) quartets. The planar associations may be unimolecular (from self-association of a single strand of DNA) or bimolecular (from the association of two or more separate strands of DNA).

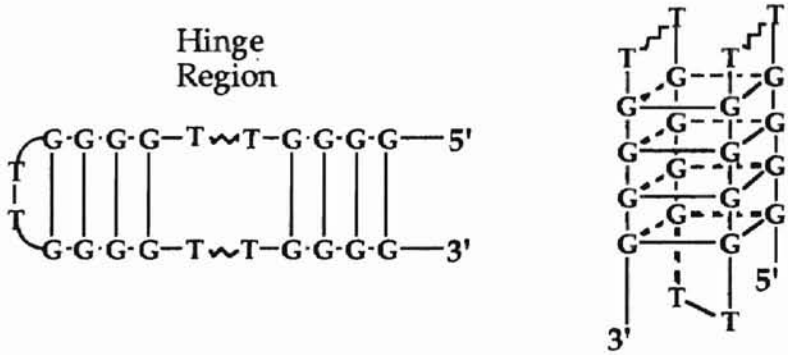


Figure 2. An example of the intramolecular folding of a telomere sequence into a G-quartet motif. The telomeric overhang folds into a hairpin, then hinges at the inner thymine (T) residues allowing the guanosine (G) to self-associate into a square planar array through Hoogsteen binding of four guanosine residues.[7]

Guanine is the most hydrophobic of the nitrogenous bases, while thymine is the most hydrophilic. These properties are thought to play a role in the folding of the oligonucleotide into the G-quartet structure. The thymine acts as a hinged region allowing the guanosine residues to come into contact with each other to establish Hoogsteen hydrogen bonding. Once the structure is folded and stabilized centrally by a monovalent salt cation, the hydrophobic guanosine residues are protected inside the structure. The sugar-phosphate backbone is exposed on the outside of the complex. The hydrophilic thymine "hinges" protect the stacked guanosine residues on each end, creating a very stable structure in the cell's aqueous environment.

Some studies have suggested that the specific associations of these guanosine repeating units are determined by the physiological salt concentration, especially those containing K^+, within the cell as the cell cycle progresses.[7] The cations stabilize the quartet structure by coordinating with the four carbonyls at the center of the quartet. Monovalent salts involving Na^+ and K^+ lend the most stability to the G-quartets in structural studies, and the concentration of K^+ in the cell is thought to be the main biological control *in vivo*.[7] Early in the cell cycle, the concentration of K^+ and DNA are both relatively low, encouraging the unimolecular folding of the guanosine repeating units. This is thought to protect the DNA from exonucleases[8] and enable telomeric binding proteins to recognize their targets.[9] As the cell cycle continues, the cellular levels of K^+ increase, encouraging bimolecular association of the guanosine repeats.[7] Bimolecular associations are thought to play an important role in the pairing of chromatids, the genetic exchange between chromosomes, and the control and association of chromosomes throughout meiosis and mitosis.[5] Unimolecular associations and the

study of their role in telomeric DNA is by far the most widely studied area of G-quartet structures.

Telomeric Sequences and the Guanosine Quartet Model

Due to the end replication problem of DNA, first proposed by Watson in 1972, chromosomes have an overhang of several bases on the 3' end. These telomeric overhangs have been found to contain very similar sequences, regardless of their genetic source. The most common motif is a repeating unit of three or more guanosine residues separated by two or more thymidine residues.

5'-3' telomeric repeat	organism (classification)
d(TTGGGG)	*Tetrahymena* (ciliated protozoa)
d(TTTTGGGG)	*Oxytriciha* (ciliated protozoa)
d(TTTTGGGG)	*Stylonychia* (ciliated protozoa)
d(TTAGGG)	*Neurospora* (filamentous fungi)
d(TTAGGG)	*Didymium* (filamentous fungi)
d(TTAGGG)	*Trypanosoma* (kinetoplastid protozoa)
d(TTTTAGGG)	*Chlamydomonas* (alga)
d(TTTAGGG)	*Arabidopsis* (higher plant)
d(GGTGTAC)	*Candida guillermondii* (budding yeast)
d(TTAGGC)	*Ascaris lumbricoides* (nematode)
d(TTAGG)	*Bombyx mori* (insect)
d(TTAGGG)	*Homo sapiens* (mammal)

Table 1. Known telomeric sequences from several organisms.[10]

When several G-quartet units are stacked on top of one another, the glycosidic linkages stagger in the anti and syn conformations, creating major and minor grooves

along the outer edges of the structure.[11] It is believed the major and minor grooves formed along the structure are important in enzymatic recognition.[7]

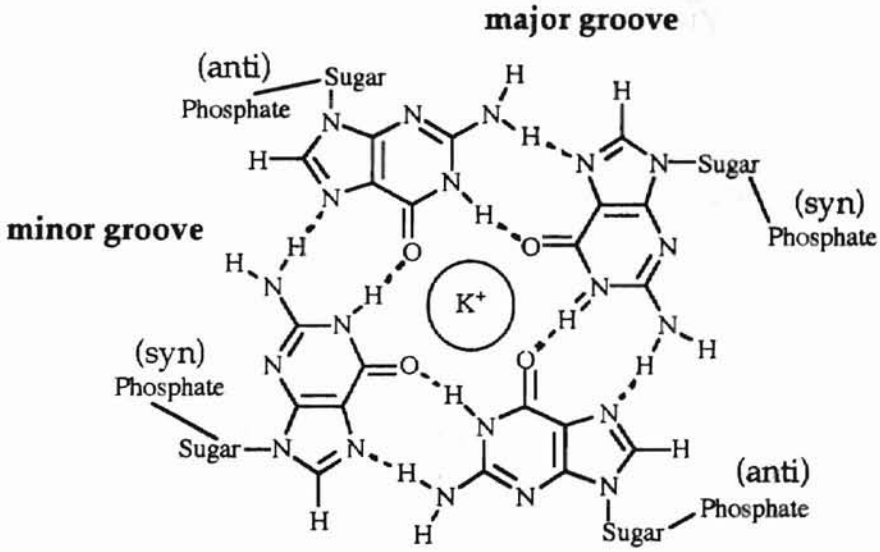


Figure 3. Once folded into the planar structure, the G-quartet is stabilized by a monovalent salt cation, usually K^+. The sugar phosphate backbone is exposed to the aqueous environment.[11]

While 7-deazaguanine is capable of normal Watson-Crick base pairing when incorporated into an oligonucleotide,[12] it cannot form the Hoogsteen binding required for G-quartet formation. The formation of G-quartets is further discouraged by proton-proton repulsion, making 7-deazaguanosine a possible tool in determining if this structure is required for enzymatic recognition, such as telomerase activity. Telomerase studies were the primary reason behind this investigation into a better synthesis for 7-deazaguanosine. Since 7-deazaguanosine only differs from guanosine by one atom and does not affect normal DNA duplex structure, it will be an important tool to answer many questions dealing with the complex structures and activities involving guanosine.

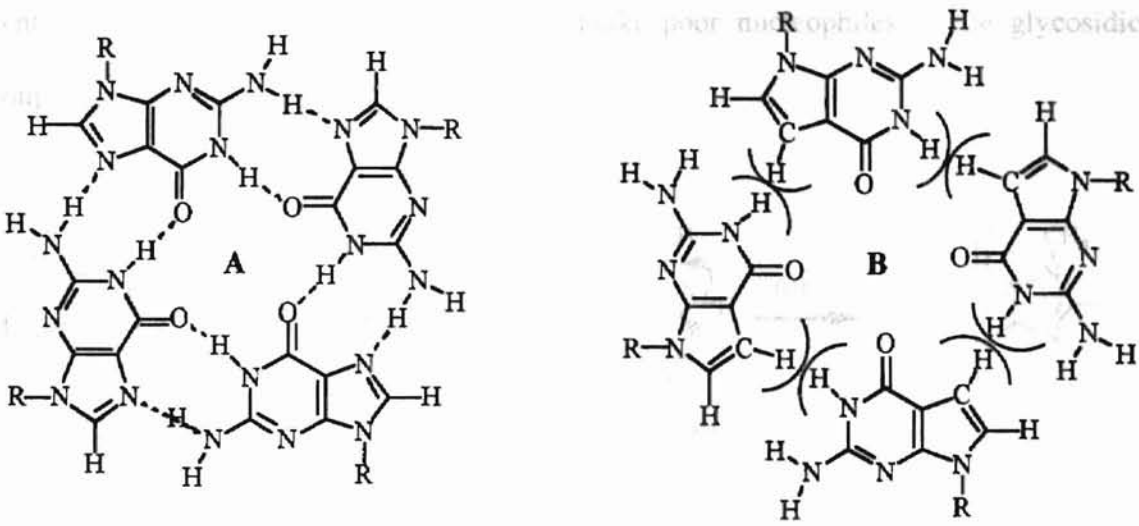


Figure 4. A. Hoogsteen base pairing requires hydrogen bonding between the N-7 of the imidazole ring of one guanosine and the pyrimidine amino group of another. **B.** In 7-deazaguanosine, the quaternary structure is discouraged by proton-proton repulsion and a lack of necessary hydrogen bonding sites.

Special Properties of 7-Deazapurine

Another important structural feature of 7-deazapurines is the increased stability of the N-glycosidic bond.[12] Replacement of N-7 by a carbon prevents methylation at this crucial position. This makes 7-deazapurine nucleosides much more resistant to cleavage by enzymatic and chemical methods. This resistance to cleavage could be exploited as a useful tool for investigating enzyme mechanisms as well as complex structures. By selectively substituting 7-deazaguanosine for guanosine in various oligonucleotides, this property could provide researchers with specific sequences resistant to nuclease activity. Decreased nucleophilicity of the pyrrolic nitrogen also plays a role in stabilizing the glycosidic bond.[13] This decreased nucleophilicity offers a challenge when preparing

synthetic 7-deazapurines, as they typically make poor nucleophiles in the glycosidic coupling reactions.

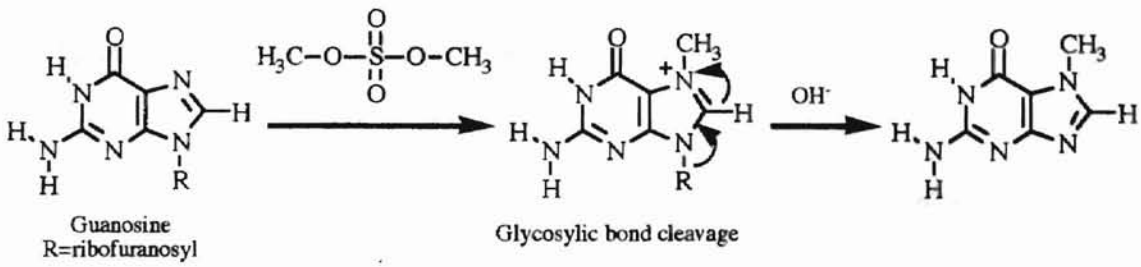


Figure 5. Glycosidic bond cleavage usually involves methylation at the N-7 position of the purine ring. In 7-deazapurines, this step cannot take place, resulting in a high resistance to cleavage.

Previous Syntheses of 7-Deazaguanosine and Similar Pyrrolo[2,3-*d*]pyrimidines

Several synthetic methodologies have been used to prepare 7-deazaguanosine and related (β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine derivatives in the past. To date, most methods involve the use of ribosyl halides (such as **9**) for the synthesis. Ribosyl halides effectively select for the β-anomer during the synthesis.

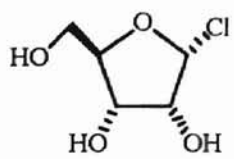


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Figure 6. An example of a typical ribosyl halide.

One of the best known methods for the synthesis of 7-deazaguanosine with ribosyl halides utilizes phase transfer chemistry and chlororibofuranosyl **11** to produce modest yields of the deoxy analog of 7-deazaguanosine **12**.[14] A chlorinated analog of 7-deazaguanine **10** is used as the precursor.

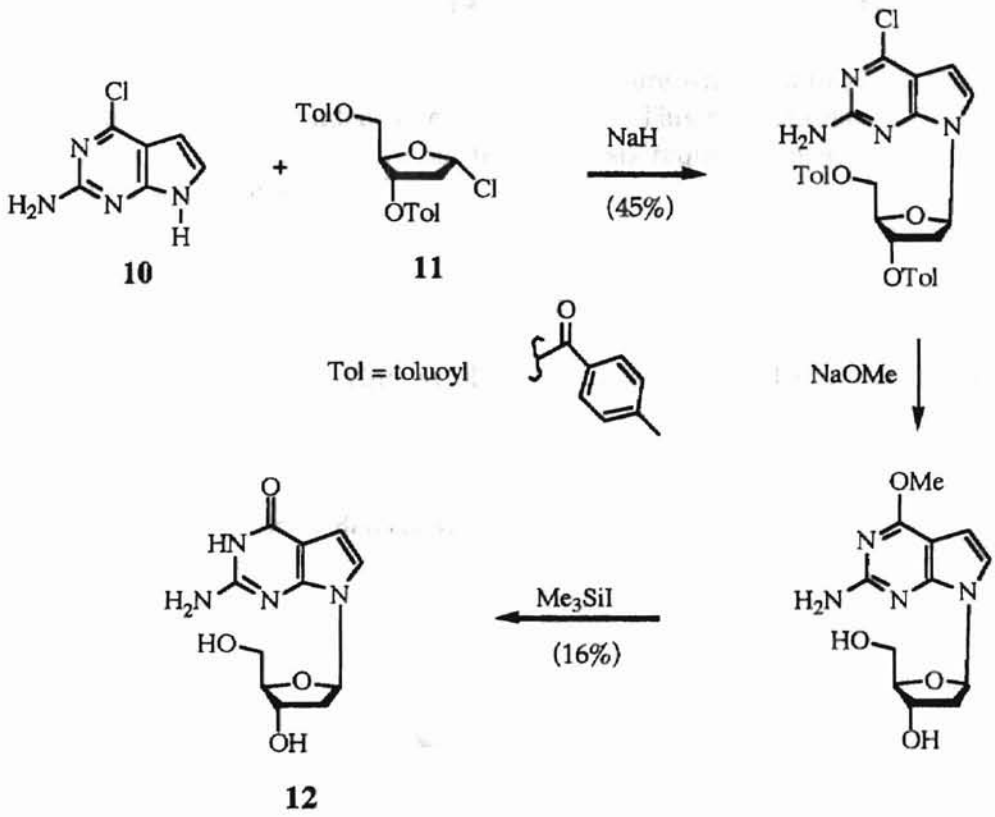


Figure 7. Phase transfer chemistry utilizing chlorosugars produces a modest yield of 7-deazaguanosine after several steps.[14]

Use of a sodium salt glycosylation procedure with α-bromoribofuranosyls, **13**, and brominated 7-deazaadenine **14** gave a high yield, regioselective and stereospecific synthesis of (β -D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine **15**.[15]

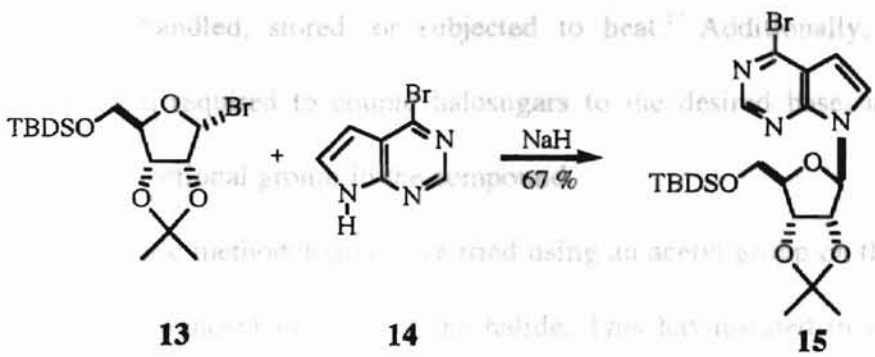


Figure 8. A sodium salt glycosylation procedure was used to synthesize a precursor of 7-deazaadenosine in high yield. This reaction has been used for 7-deazaguanine as well, but the synthesis requires several steps after the initial sugar coupling.[15]

Mercuric oxide has also been used to couple bromosugars to similar pyrrolo[2,3-*d*]pyrimidine derivatives.[16] This synthesis uses a trimethylsilyl derivative **16** and protected bromosugars **17** for the ribosylation step. The result is a low yield of a chlorinated 7-deazaadenosine derivative **18**.

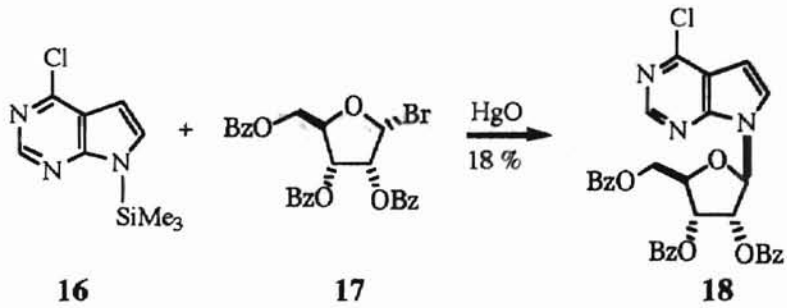
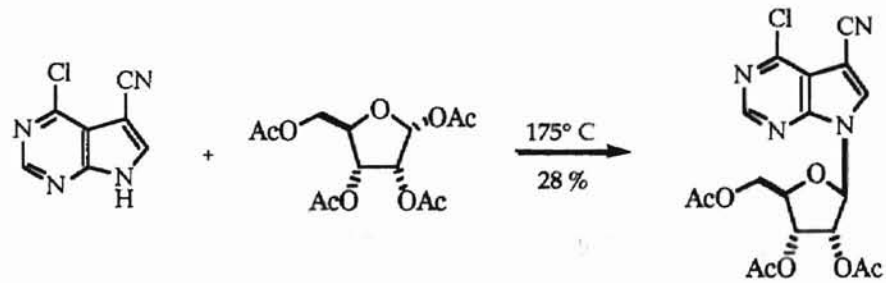


Figure 9. Trimethylsilyl pyrrolo[2,3-*d*]pyrimidines and mercuric oxide results in forming 7-deazanucleosides in low yields.[16]

All of these methods have resulted in the desired nucleoside, but ribosyl halides are required as precursors. While ribosyl halides may offer excellent regioselectivity for the β-anomeric product, they also have serious drawbacks. Due to the instability of the halosugars, they are difficult to manipulate in the laboratory. Halosugars rapidly

decompose when handled, stored, or subjected to heat.[17] Additionally, harsh basic conditions are often required to couple halosugars to the desired base, and this may degrade the other functional groups in the compound.

Some synthetic methodologies have tried using an acetyl group on the 1' position of a protected ribofuranosyl in place of the halide. This has resulted in a much more stable sugar, making the coupling more difficult. Consequently, yields of the desired nucleoside are low. This reaction often utilizes a fusion procedure. In a fusion reaction the selected pyrrolo[2,3-*d*]pyrimidine is ground together with a protected furanose, and the two compounds are heated in an oil bath until they fuse together. An acidic catalyst is often required for reaction to occur. The fusion procedure was used to couple 1',2',3',5'-tetra-*O*-acetyl-β-D-ribofuranose to several different pyrrolo[2,3-*d*]pyrimidine derivatives.[2]



20 **19** **21**

Figure 10. The fusion procedure uses a more stable ribosyl precursor, but the synthesis gives low yields of nucleoside.[2] The reported yield is based on unrecovered starting material, not purified nucleoside.

Fusion reactions are facilitated by the presence of electron-withdrawing substituents on the pyrrolo[2,3-*d*]pyrimidine. Halogen substituents on the purine seem to increase the likelihood of a successful coupling. This procedure has been used to

successfully prepare **1**, **5**, and **6**.[2] Structural intermediates in these three pyrrolo[2,3-*d*]pyrimidine syntheses are very similar to 7-deazaguanine, but the resulting yields are too low for preparation of the nucleoside in useful quantities. Yields of 28% were reported for the reaction above, but this was based on unrecovered starting materials, not the purified nucleoside.

Carbohydrate chemistry, specifically the work of Fraser-Reid and his co-workers,[18] has inspired the use of *n*-pentenyl ribosides for purine nucleoside synthesis.[17] Pentenyl ribosides offer an extremely stable sugar moiety that may be manipulated with various protecting groups to aid in the synthesis of a β-nucleoside in good yields.

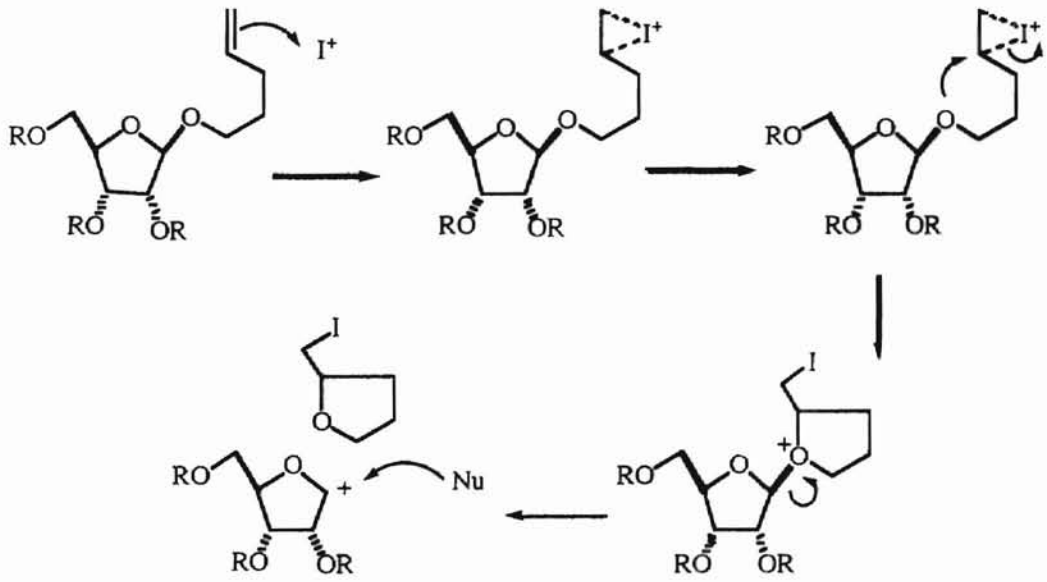


Figure 11. Pentenyl ribosides form a carbocation at the 1' position with the addition of I^+.[18]

Activation of pentenyl ribosides is carried out under neutral or mildly acidic conditions using I^+ to promote carbocation formation in the 1' position. This facilitates ribosylation of the purine base. Until they are activated with I^+, pentenyl ribosides are

extremely stable to many chemical manipulations and may be stored indefinitely until needed. This provides an easily controlled synthesis of ribonucleosides with reasonable yields.[17] Our investigation looked into the possibility of utilizing protected pentenyl ribosides for the synthesis of 7-deazaguanosine. This methodology was of interest due to the stability of the pentenyl sugars, enabling preparation of 7-deazaguanosine quickly from stable precursors on an as-needed basis.

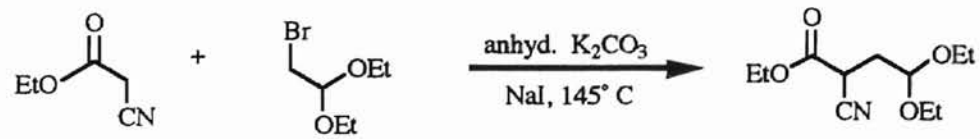
CHAPTER II

RESULTS AND DISCUSSION

Our initial research methodology sought to prepare 7-deazaguanosine by the existing synthesis of Davoll.[19] Once the purine base was available, ribosylation of the base with protected pentenyl ribosides would afford 7-deazaguanosine. By carefully choosing the protecting groups on the pentenyl riboside, it was hoped that the synthesis would be stereospecific and yield the β anomer in useful quantities.

Synthesis of 7-Deazaguanine

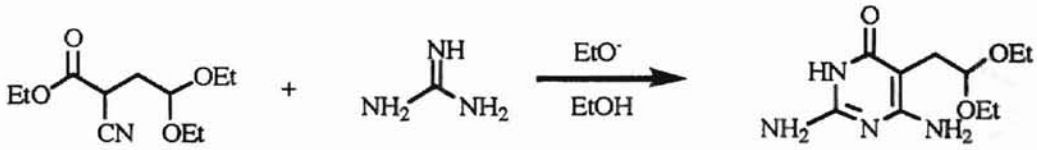
The purine base was prepared in three steps. In the first step, ethyl cyanoacetate (**22**) was alkylated with bromoacetaldehyde diethyl acetal (**23**) to give ethyl 2-cyano-4,4-diethoxybutanoate (**24**). This reaction proceeded in low yield, but afforded enough of the necessary precursor to continue the purine synthesis.



22 **23** **24** (17%)

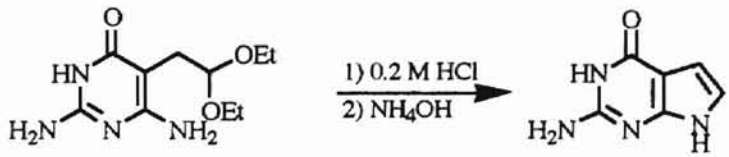
The pyrimidine ring portion of 7-deazaguanine was formed by reacting **24** with guanidine in a base-catalyzed condensation reaction.[19] This reaction gave a much better yield than the initial step, but the overall yield of pyrrolo[2,3-*d*]pyrimidine was low. It

was necessary to scale up the experiments to obtain enough 2,4-diamino-5-(2,2-diethoxyethyl)pyrimidin-6-one (**25**) for the remainder of the syntheses.



24 **25** (45%)

The final step in forming the pyrrolo[2,3-*d*]pyrimidine was an acid-catalyzed ring closure of the isolated diamino compound. This was easily accomplished and gave very high yields (88-93%) of 2-aminopyrrolo[2,3-*d*]pyrimidin-4-one **26**, the target 7-deazaguanine. It was discovered that this final ring closure occurred spontaneously if excess acid was used in the workup of **25**. The first time the synthesis of **25** was attempted, **26** was confirmed as the product in 71% YIELD by ^{1}H NMR, GC-MS, and FAB. However, **26** was obtained in a much lower yield (41%) if **25** was isolated and recrystallized first.

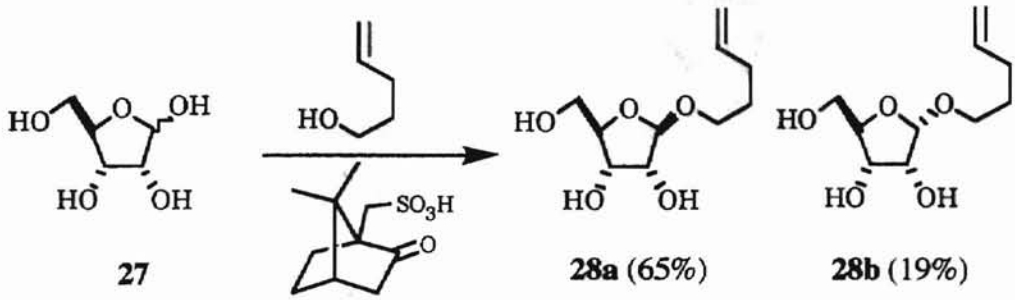


25 **26** (93%)

Synthesis of the Ribosyl Precursors

Once **26** was prepared, attention was turned to the synthesis of an appropriate pentenyl ribose moiety. The synthesis started with D-ribose (D-*erythro*-pentofuranose) **27** as the precursor. The first step involved protection of the anomeric carbon by an acid-

catalyzed glycoside formation using an excess of 4-penten-1-ol, according to the procedure of Fraser-Reid and co-workers.[18] This method utilizes (±)-10-camphorsulfonic acid as the catalyst, and follows the Fischer glycosylation method.



27 **28a** (65%) **28b** (19%)

The glycosidic coupling resulted in the formation of both pent-4-enyl β-D-*erythro*-pentofuranoside (**28a**) and pent-4-enyl α-D-*erythro*-pentofuranoside (**28b**). These anomers were easily separated using flash chromatography. Due to the fact that the sugar moiety forms a planar carbocation during the purine coupling step, both anomers could be used for the purine ribosylations. The yield of the β anomer was much higher than the α form (77:23, β:α isolated), and most of our coupling attempts used **28a** as the preferred precursor.

Enhancing the Stereoselectivity of the Ribosylation

After the pentenyl side chain was added to **27**, selection of a protecting group for the remaining alcohol substituents was made. We chose the benzoyl (Bz) protecting group to assist in the stereoselectivity of the β anomer of 7-deazaguanosine **3**. As the carbocation is formed, a lone pair of electrons on the oxygen of the benzoyl carbonyl stabilizes the positive charge, effectively providing steric hindrance on the underside of

the anomeric carbon. This allows nucleophilic attack from the topside of the anomeric carbon only, effectively selecting for the β anomer as the major product.[17]

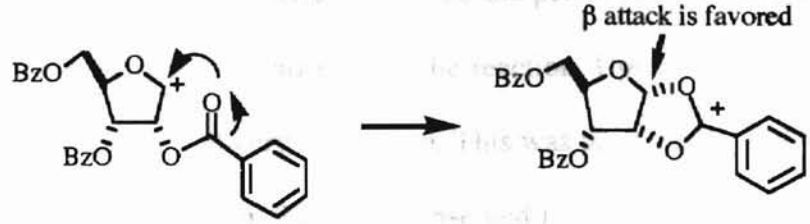
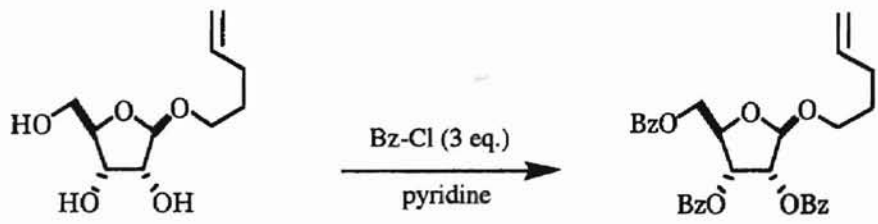


Figure 12. Use of a benzoyl protecting group on the activated pentenyl ribose promotes β attack on the carbocation by the nitrogenous base.[17]

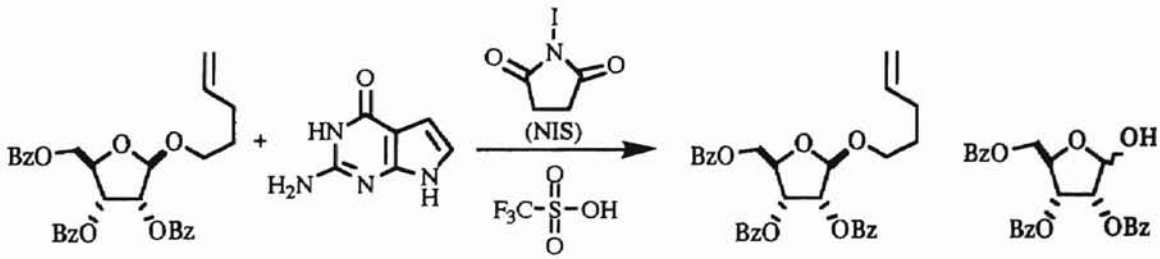
Our synthesis of pent-4-enyl 2',3',5'-tri-*O*-benzoyl-β-D-*erythro*-pentofuranoside (**29**) followed the work of Chappeau and Marnett,[17] who first utilized this protecting group in the steroeselective synthesis of purine nucleosides. The synthesis of **29** utilized a benzoyl chloride for esterification of the remaining alcohol groups. The pentenyl side chain was not affected by the addition of the benzoyl group, and the fully protected sugar was obtained in a good yield.



28a **29** (80%)

The Initial Ribosylation Reactions

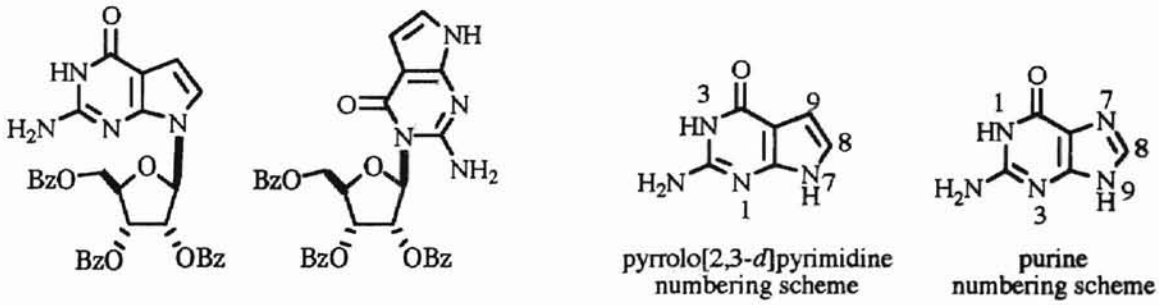
Once the protected sugar was purified, the first coupling procedure was undertaken. The initial attempt used a combination of *N*-iodosuccinimide (NIS) and trifluoromethane sulfonic (triflic) acid to activate the pentenyl riboside. Acetonitrile was used as the solvent to promote ionization in the reaction. Upon the addition of NIS and triflic acid, the reaction turned very dark green. This was unexpected, and after removing the solvent, no coupling between the purine base and the sugar could be detected. Thin-layer chromatography (TLC) of the crude reaction mixture revealed mostly unreacted **29** and its hydrolysis product **30**. The presence of unreacted **26** could not be detected, but there were several new spots of less polar substances present. The reaction was repeated, and the results were the same. The crude reaction mixture was worked up and purified by column chromatography, but no detectable amount of the desired product was formed. The ^{1}H NMRs of the separated compounds showed protons from the unreacted sugar, but none of the signals from the purine were observed. After the initial coupling attempt, the only detectable product was 2',3',5'-tri-*O*-benzoyl-D-*erythro*-pentofuranoside (**30**).



29 **26** **29** **30**

Problems Encountered in Ribosylation Reactions

One problem in coupling 7-deazaguanine with a sugar is the lack of control over which nitrogen is involved in the nucleophilic attack on the carbocation. It has been reported that N-3 actively participates in the nucleophilic attack on a ribosyl halide.[20] The numbering system of a pyrrolo[2,3-*d*]pyrimidine and a purine differ. When acting as a purine base, the accepted numbering system for a purine is used (N-7 is referred to as N-9). This causes a lot of confusion when dealing with pyrrolo[2,3-*d*]pyrimidines.



26

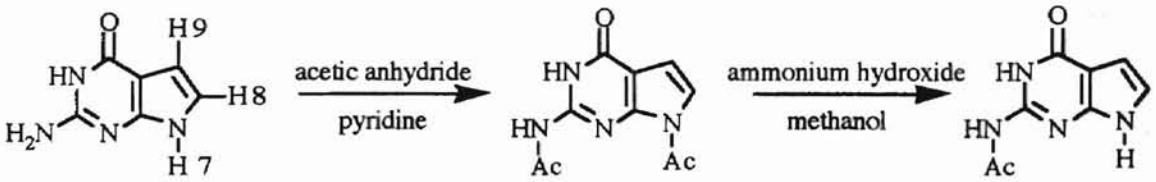
Figure 13. There are two nucleophilic nitrogens in **26**, N-7 and N-3.[20]

Acetylation of 7-Deazaguanine

To help eliminate the possibility of mixed products in our reactions, we prepared an acetylated analog of 7-deazaguanine by reacting **26** with acetic anhydride in pyridine.[21] This should discourage nucleophilic attack by N-3, leaving only N-7 available for coupling to the pentenyl riboside.

Initial acetylation occurs at two positions on **26**, placing an acetyl group (Ac) on both the C-2 amino substituent and the N-7 of the pyrrolo[2,3-*d*]pyrimidine forming 2-acetamido-7-acetylpyrrolo[2,3-*d*]pyrimidin-4-one (**31**). A second reaction selectively

removes the acetyl group on N-7, resulting in 2-acetamidopyrrolo[2,3-*d*]pyrimidin-4-one (**32**). The presence of this acetyl group should discourage the competing nucleophilic attack.

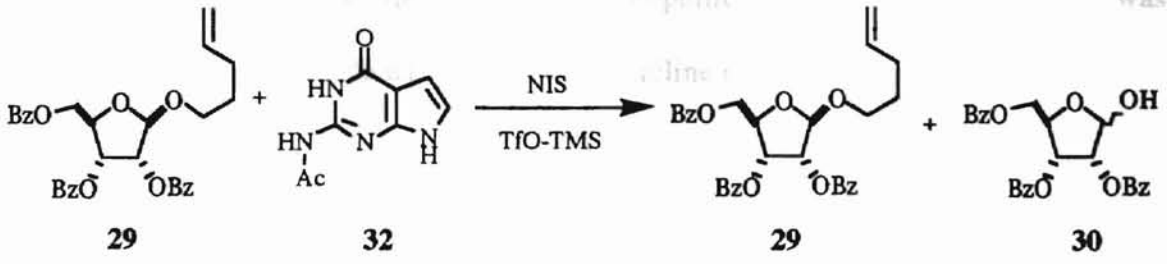


26 **31** (81%) **32** (88%)

Figure 14. It is possible to selectively acetylate **26**, but two steps are required. The acetyl group on the ring nitrogen is easily removed in a dilute basic solution.[21]

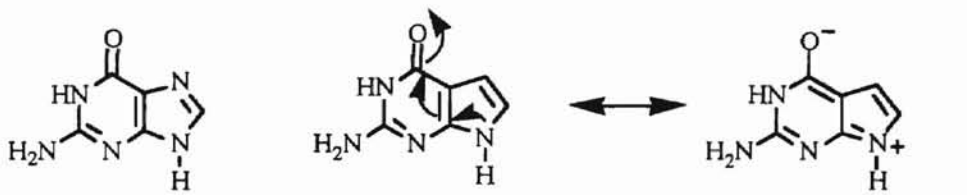
It was confirmed that N-7 is the second site of acetylation on the ring by observing the ^{1}H NMR splitting patterns of H-9 and H-8. The H-7 proton splits the signals of H-9 and H-8 into four peaks each (two doublets of doublets). After initial acetylation, these signals contained only two peaks each (two doublets), indicating the proton on N-7 was replaced by the acetyl group. When the acetyl group was selectively removed, the signals returned to the original doublet of doublets pattern. This change in multiplicity is very useful in determining if N-7 is involved in new bond formation and provides a good diagnostic signal to confirm a glycosylation at the N-7 position.

Once **32** was prepared, another ribosylation with **29** was attempted. The reagents were the same except trimethylsilyl triflate (TfO-TMS) was substituted for triflic acid. It was hoped this would create a more controlled source of I^+.[22] Upon addition of NIS and TfO-TMS, the reaction turned black.



29 **32** **29** **30**

No trace of **32** could be detected by TLC, and the reaction was quenched. The only product present was an anomeric mixture of 2',3',5'-tri-*O*-benzoyl-D-*erythro*-pentofuranoside (**30**). No coupled products could be detected. Chappeau and Marnett reported **30** as the major product when guanosine was used in this reaction.[17] They suggested that guanine was a very weak nucleophile. Our results suggest that **26** may be an even weaker nucleophile, due to the fact that no ribosylated purines were detectable.



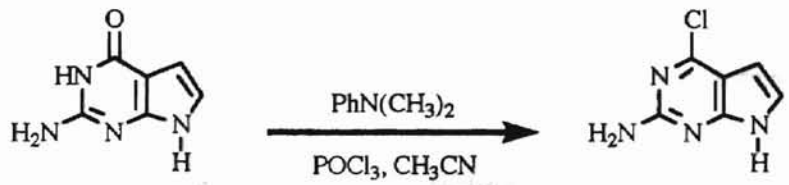
guanine **26** poor nucleophilic character

Figure 15. The resonance structure for guanine helps to explain its poor nucleophilic character. The electron-withdrawing affect of the carbonyl group places a partial positive charge on N-7.

Attempts to Increase the Nucleophilic Character of 7-Deazaguanine

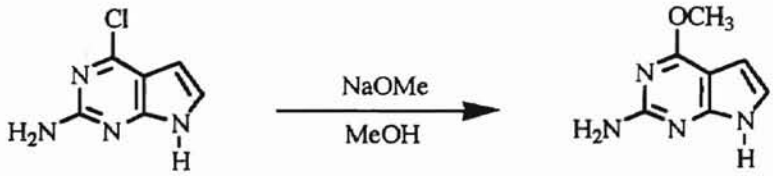
In an attempt to increase the nucleophilic character of **26**, the carbonyl oxygen of **26** was replaced with a chloride atom to give 2-amino-4-chloropyrrolo[2,3-*d*]pyrimidine (**10**). It was hoped that this substitution would reduce the electron-withdrawing affect of

the carbonyl group, and increase the nucleophilic character of N-7. This was accomplished by reacting **26** with *N,N*-dimethylaniline in $POCl_3$.[23]



26 **10** (55%)

We also prepared 2-amino-4-methoxypyrrolo[2,3-*d*]pyrimidine (**33**), a methoxy analog of **13**, in an attempt to further increase the nucleophilic character of N-7. By replacing the chlorine atom with a methoxy group, electron density is donated into the pyrimidine ring system. This was accomplished by reacting **10** with methanolic sodium methoxide.[4]



10 **33** (69%)

Orthoamide Formation

To help reduce side reactions, we decided to adjust the protecting groups on the ribose. The use of the benzoyl group to aid in regioselectivity for the β-anomeric product introduces another problem in the synthesis of nucleoside analogs. Nucleophilic attack may occur at the benzylic carbon. This attack would form an undesirable orthoamide side product. [24]

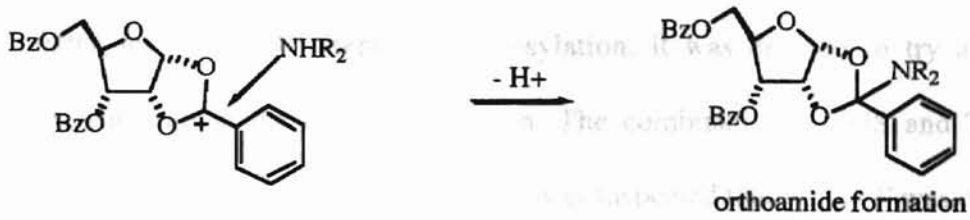
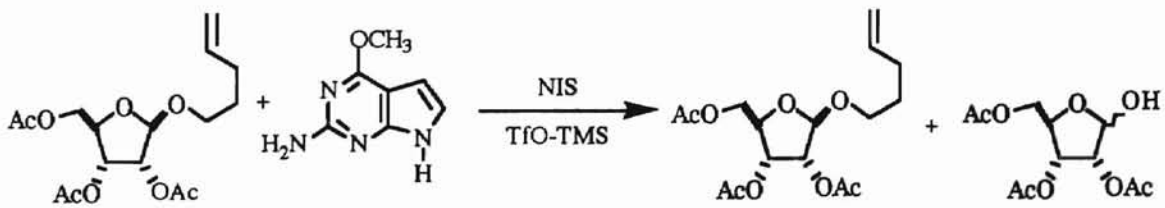


Figure 16. The use of a benzoyl protecting group, while aiding in stereoselectivity of the β anomer, may lead to an undesirable orthoamide product. [24]

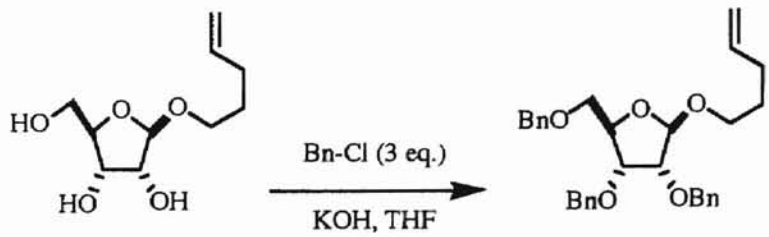
To help minimize orthoamide formation, we replaced the benzoyl (Bz) group with an acetyl (Ac) protecting group. This should still afford stereoselectivity for the β anomer without providing the electron density that stabilizes the carbocation formed β to the anomeric carbon. By cutting down on undesirable bond formations, it was hoped we could obtain a detectable amount of our coupled nucleoside. We prepared pent-4-enyl 2',3',5'-tri-*O*-acetyl-β-D-*erythro*-pentofuranoside (**34**) by reacting **28a** with acetyl chloride. A coupling between **33** and **34** was attempted using NIS and TfO-TMS, but the results of the reaction were the same as all the other attempts. The only detectable major product was an anomeric mixture of 2',3',5'-tri-*O*-acetyl-D-*erythro*-pentofuranoside (**35**).



34 **33** **34** **35**

Coupling Reactions with Iodonium Dicollidine Perchlorate (IDPC)

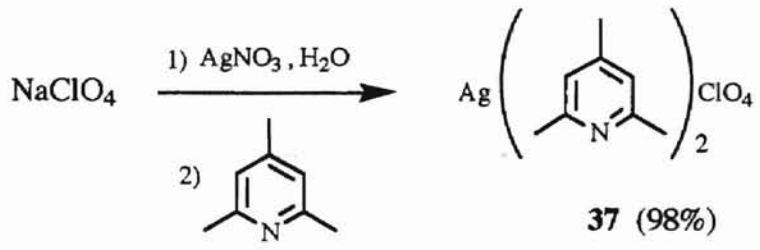
After several failed attempts at ribosylation, it was decided to try a different source of iodonium in the coupling reaction. The combination of NIS and TfO-TMS provides a good source of I^+ in the reaction. It was suspected that excess I^+ was destroying the 7-deazapurine base in solution. Since 7-deazaguanine and its analogs contain a reactive double bond between C-7 and C-8, it is possible that excess I^+ could be reacting with this functional group. Iodonium dicollidine perchlorate (IDPC) has been used successfully as a source of iodonium in pentenyl riboside couplings.[17] This reagent cannot be used when an acyl group is present at the 2' position of the sugar, but use of a benzyl (Bn) group helps to enhance its reactivity.[17] In order to utilize this I^+ source, we prepared pent-4-enyl 2',3',5'-tri-*O*-benzyl-β-D-*erythro*-pentofuranoside (**36**) by reacting **28a** with benzyl chloride and potassium hydroxide (KOH) in tetrahydrofuran (THF).[25] By using the benzyl protected ribose, stereoselectivity for the β anomer is sacrificed, but the coupling reaction takes place under neutral conditions. It was hoped the milder reaction conditions would result in a successful coupling between **36** and our purine analogs.



28a **36** (80%)

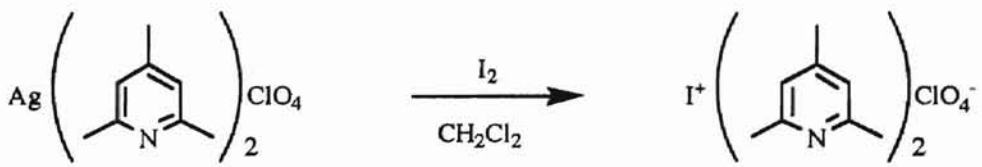
The Synthesis of IDPC

Our IDPC was prepared in two steps by the method of Lemieux and Morgan.[26] In the first step, sodium perchlorate and silver nitrate were combined with 2,4,6-collidine to give silver di-2,4,6-collidine perchlorate (**37**). This reaction gave a good yield.



37 (98%)

The second step replaced the silver with an I^+ ion by reacting **37** with iodine in methylene chloride, forming iodonium di-2,4,6-collidine perchlorate (**38**). The second step resulted in a low yield, possibly due to the quality of our iodine crystals. Lemieux and Morgan used powdered anhydrous iodine, while we used fine iodine crystals. The second reaction is sensitive to the presence of water, so it is quite possible our iodine crystals contained some moisture.



37 **38** (38%)

Once **38** was prepared, a titration was performed to determine the amount of free iodonium in the compound. The titration used a 0.13 M solution of sodium iodide in 2-propanol to form I_2 with the free I^+ ion, then the iodine solution was titrated with an aqueous solution of 0.1 M sodium sulfite.

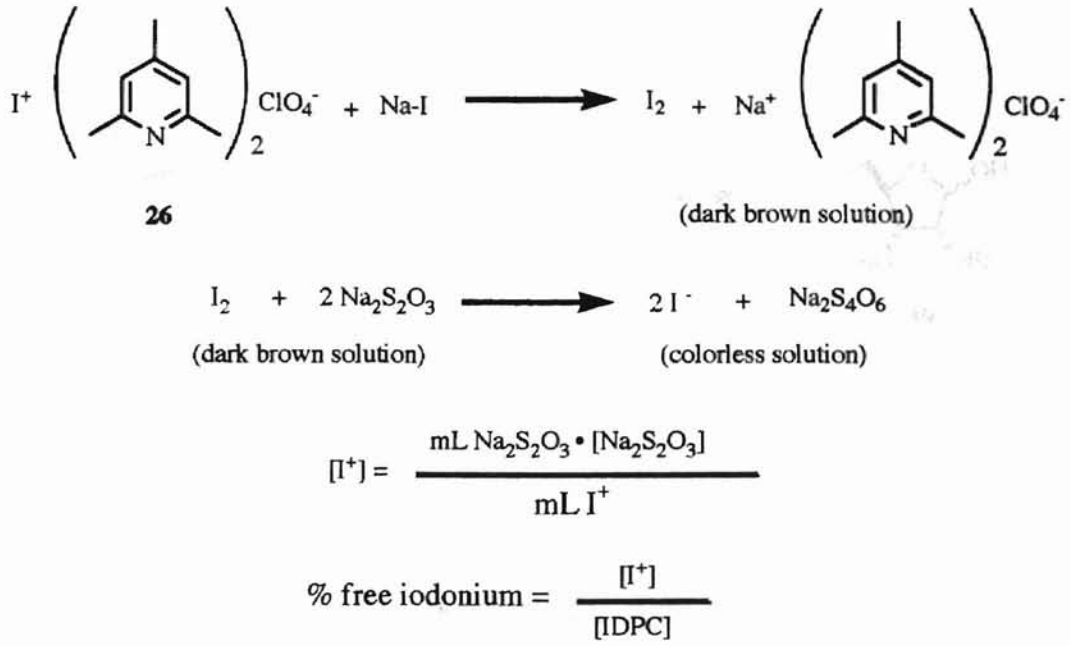
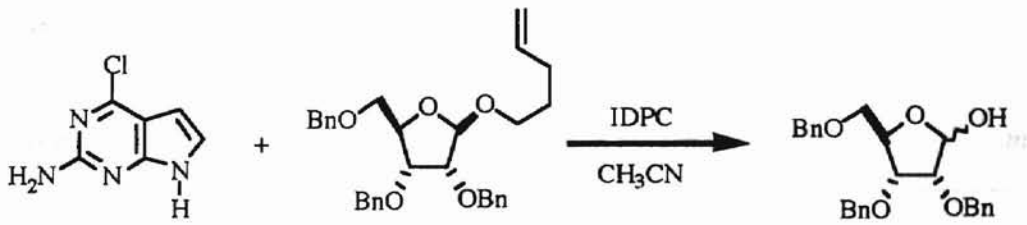


Figure 17. The concentration of free I^+ in our synthetic IDPC was determined by titration.

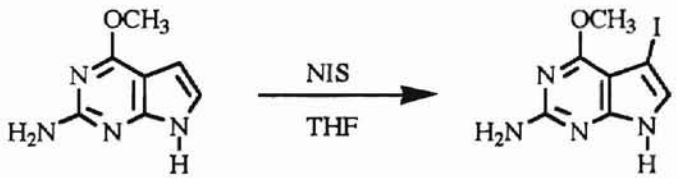
Using this method, our IDPC contained 90% free I^+ in solution. The titration was repeated with *N*-iodosuccinimide, which contained 68% free I^+ in solution. This caused some concern, since it was suspected that excess I^+ was destroying the purine base during the coupling reactions. It was hoped that the neutral reaction conditions would prevent the base from reacting with the I^+. We attempted a coupling between **10** and **36** using IDPC in acetonitrile. The reaction turned dark green upon the addition of IDPC. After thirty minutes, new spots were detected on the TLC plate between the two starting materials, but these appeared to be traces of 2',3',5'-tri-*O*-benzyl-D-*erythro*-pentofuranoside (**39**). The plates indicated most of our starting material was still present. Another equivalent of IDPC was added, and the reaction was allowed to stir for an additional 48 hours. No change was noted.



10 **36** **39**
(trace amounts)

Attempted Halogenation of 2-Amino-4-methoxypyrrolo[2,3-*d*]pyrimidine

It was decided to investigate the possibility of the I^+ halogenating the purine base in these reactions. An experiment was set up with **33** and NIS in THF. The reaction was protected from light. Upon addition of the NIS, the reaction turned dark green. The reaction was followed by GC-MS to determine if an iodine was substituted onto **21**.



33 **40**
(trace amounts ?)

A small amount of a compound with the molecular ion $m/z = 290$ was detected by GC-MS, matching the expected molecular weight of 2-amino-7-iodo-4-methoxy-pyrrolo[2,3-*d*]pyrimidine (**40**). Upon closer inspection of the mass spectrum, no peak for M-127 (loss of iodine) was observed. Since aromatic halides give a strong M-X peak

when the halogen is directly attached to an aromatic ring, it is doubtful that the molecular ion corresponded to **40**.

Compound	Formula	MW	Retention time	*m/z*
26	$C_6H_6N_4O$	150	22.4 min	150
31	$C_8H_8N_4O_2$	192	34.6 min	192
10	$C_6H_5ClN_4$	168	20.5 min	168
33	$C_7H_8N_4O$	164	19.6 min	164
40*	$C_7H_7IN_4O$	290	26.0 min	290

* could not be confirmed

Table 2. GC-MS data of 7-deazapurines in this study. The presence of **40** could not be confirmed by GC-MS due to the lack of a M-I peak. All of the deazapurines in this study gave distinct molecular ions, with the strongest peak at $m/z = 149$.

The reaction was quenched, and the organic compounds were extracted into ethyl acetate. After drying the organic layer, it was concentrated to a brown residue with black solids. After purifying the mixture by flash chromatography, we obtained a yellowish brown solution that turned light green upon concentration. This residue was dissolved and analyzed by HPLC to reveal nine different compounds. Of these nine compounds, only one displayed a UV spectrum similar to **33** with a bathochromic shift of 10 nm expected for the halogenated compound. This information was not enough to confirm that **40** was a definite product of the reaction.

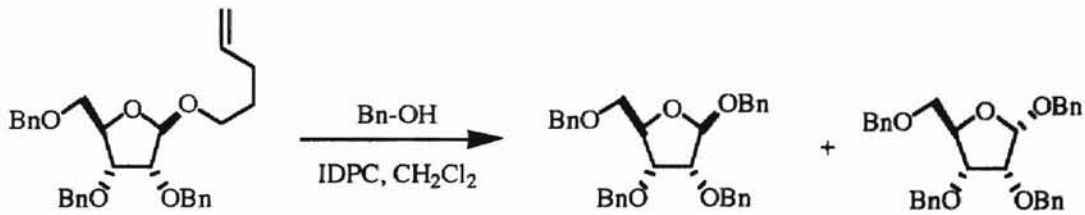
Compound	λ_{max} (Band I)	λ_{max} (Band II)	λ_{max} (Band III)
36	254 nm		
34	258 nm		
29	207 nm	274 nm	
26	217 nm	257 nm	
32	221 nm	275 nm	
10	231 nm	318 nm	
31	237 nm	263 nm	320 nm
33	223 nm	257 nm	288 nm
40	233 nm	260 nm (weak)	290 nm

Table 3. HPLC data for compounds in this study. The λ_{max} for these compounds were obtained by HPLC analysis. All of the bands had fine structure except for band II of the compound suspected to be **40**.

Due to all the side products, it was difficult to confirm the exact structure of **40**. A good separation of all the products could not be achieved on the HPLC, and obtaining enough of the suspected compound for ^{1}H NMR analysis proved to be a challenge. The NMR showed a 2:1 ratio of **33** and several new unknown aromatic signals. Because of the difficulty in the separation of the products and the number of side reactions in the mixture, it was evident that the deazapurines could not withstand the addition of the I^+ ion.

Coupling Experiments Using Other Nucleophiles

To confirm that the coupling procedures would result in a ribosylation, two additional experiments were attempted. In the first experiment, a coupling was attempted between benzyl alcohol and **36** using IDPC in methylene chloride.[18] After the addition of IDPC, the mixture turned a deep yellow. After 40 minutes the reaction was quenched. The solution was amber in color, unlike the 7-deazapurine suspensions. Only two products were observed on the TLC plate when the reaction was quenched. After removing the solvent, the resulting oil was separated into the two compounds by flash chromatography. ^{1}H NMR confirmed that the products were the two expected anomers: 1',2',3',5'-tetra-*O*-benzyl-β-D-*erythro*-pentofuranoside (**41a**) and 1',2',3',5'-tetra-*O*-benzyl-α-D-*erythro*-pentofuranoside (**41b**). The reaction was very clean, and no side products were detected. The low mass balance resulted from loses incurred during separation of the anomers.

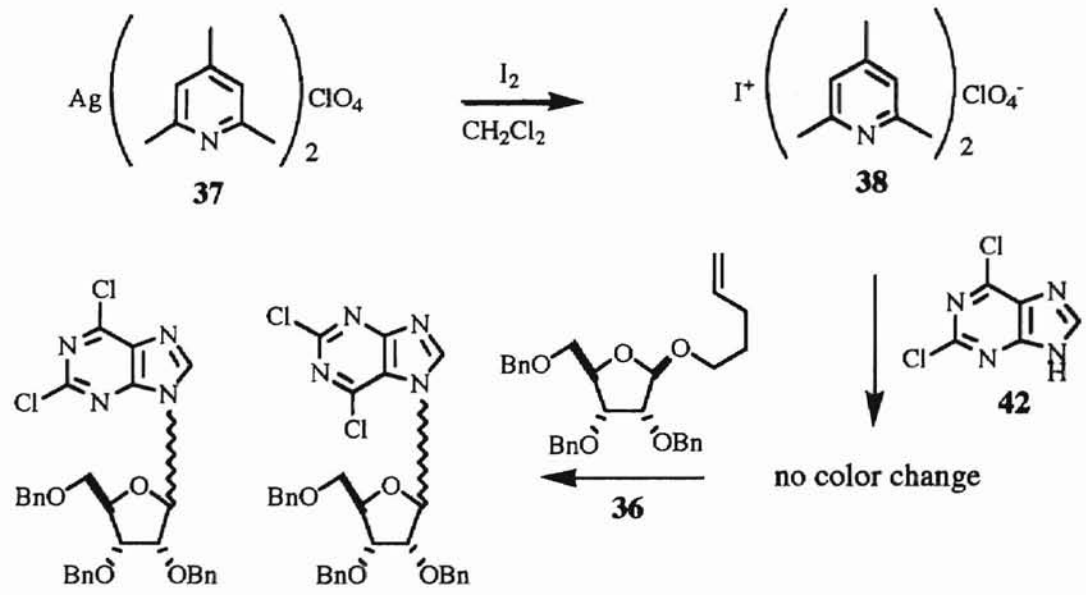


36 **41a** (22%) **41b** (6%)

The next experiment used an unrelated purine, 2,6-dichloropurine (**42**), for the nucleophile, and an *in situ* IDPC coupling with **36** was attempted.[27] This coupling scheme generates the IDPC from **37**, without having to isolate the IDPC first. The silver salts precipitate out of solution and do not interfere with the reaction. This reaction was attempted several times with all of the 7-deazapurines synthesized in this study, but the

results were always the same. The mixture turned dark green, then black after the addition of 7-deazapurine.

After dissolving **37** in acetonitrile, iodine was added directly to the mixture, and the reaction was stirred for 0.5 h. After the silver salts precipitated out of the yellow solution, **42** was added. No color change was observed. Next, **36** was introduced and the mixture was allowed to stir for an additional 4 hours. There were no color changes during the reaction. After quenching the reaction, the product was extracted into ethyl acetate and isolated as an oil. The oil was purified by flash chromatography to give a small amount of **36** (19 mg). Additionally, an anomeric mixture of the hydrolyzed carbocation was isolated along with several products that appeared to be ribosylated purines.



A FAB analysis confirmed that compounds with the molecular weight of the ribosylated purines were present. The compounds were determined to be anomeric mixtures of 2,6-dichloro-9-(D-ribofuranosyl)purine (**42**) and 2,6-dichloro-7-(D-ribofuranosyl)purine (**43**).

Attempted Hydrogenation of 7-Deazapurine

An additional experiment was set up to see if the double bond between C-8 and C-9 could be easily hydrogenated. It was apparent that this double bond was very reactive towards the iodonium ions present in the reaction. If it was possible to reduce the double bond, a coupling should be successful. The double bond should be easy to replace once the coupling took place, due to the stability of the aromatic structure in **26**. Our first attempt used 10% palladium on carbon (10% Pd/C) in methanol.[28]

Many substituent groups poison catalytic hydrogenations. An amino group will prevent the hydrogenation from taking place.[28] so we chose the acetylated 7-deazaguanine, **32**, for this reaction. The methanol was placed in a small beaker, **32** was added and the mixture was stirred. The Pd/C was added, and the reaction was placed in a sealed vessel under 60 psi H_2.

A special reaction vessel (Figure 18) was designed to allow for hydrogenation in a glass-lined pressure reactor. The stainless steel vessel had a heavy threaded lid fitted with two threaded high pressure inlets. Both of the inlets could accept high pressure lines and valves to control the gas flow. The reaction took place in a small beaker enclosed in the stainless steel vessel. This allowed a safe and controlled way to place the reaction under pressurized hydrogen gas. The hydrogen line was threaded into the inlet valve, and a dry nitrogen line or vacuum line was attached to the outlet valve as needed. This allowed us to degas the reaction vessel prior to the introduction of hydrogen and to flush hydrogen from the reactor following the reaction.

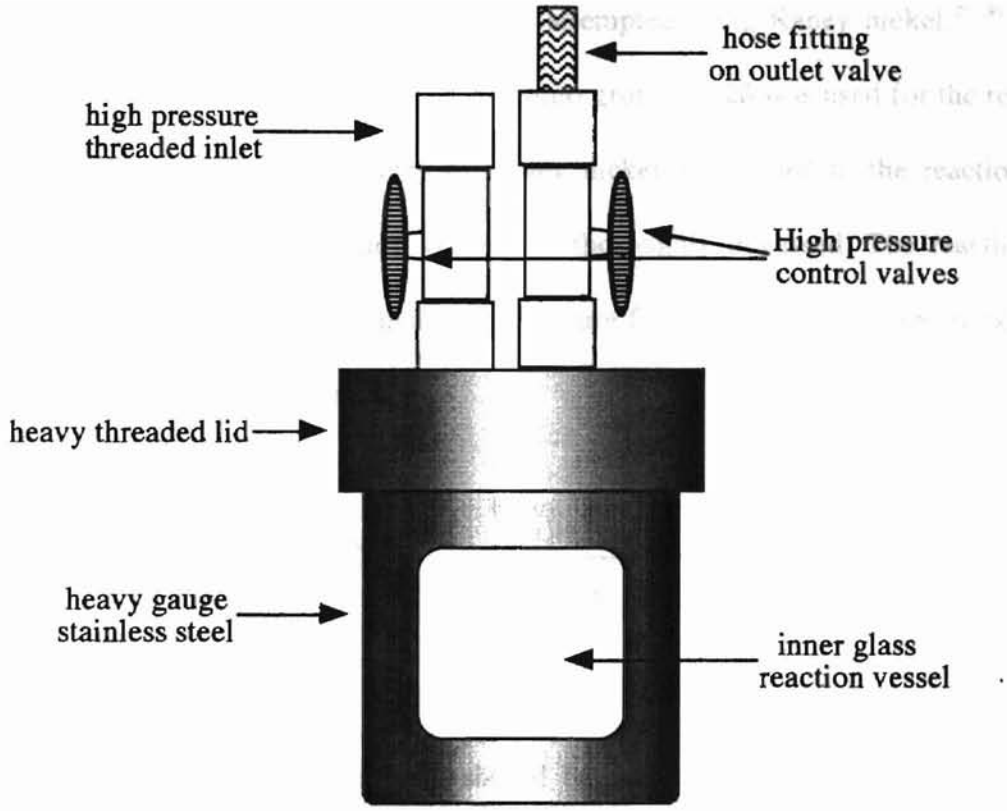
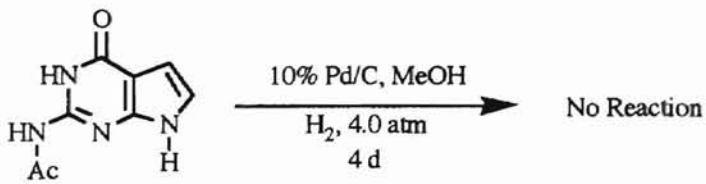


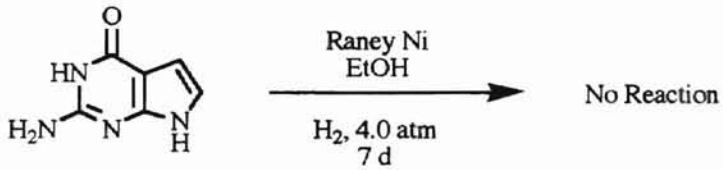
Figure 18. In order to have a safe controlled way to add pressurized H_2 to the reaction, a stainless steel reaction vessel was designed. The vessel was fabricated by the machine shop at Oklahoma State University.

Once the reaction was thoroughly degassed, the vessel was placed under a 2.7 atm (40 psi) pressure of hydrogen. After 8 h, no reaction had taken place, so the pressure was increased to 4.0 atm (60 psi). The reaction was allowed to stir under hydrogen, but no reaction could be detected after 4 d.



32

A second hydrogenation reaction was attempted using Raney nickel.[29, 30] Raney nickel is not affected by the presence of an amino group, so **26** was used for the reaction. After placing **26** in ethanol, a slurry of Raney nickel was added to the reaction. The mixture was placed in the reaction vessel and thoroughly degassed. The reaction was stirred under 4.0 atm (60 psi) of hydrogen pressure for 7 d, but no reaction took place; only starting material was detected.



26

Theoretically, the double bond should be easy to remove, but 7-deazaguanine proved to be difficult to reduce. The reaction would probably proceed under much higher pressure, but the required equipment was not available. With the wide range of hydrogenation catalysts available, an efficient one for the hydrogenation of **26** probably exists. Unfortunately, this project had to be closed without any further investigation. Since the primary goal of this research was to provide an easy synthesis of 7-deazaguaninosine from stable precursors, the addition of a dehydrogenation step would have been a major inconvenience.

CHAPTER III

CONCLUSIONS

During this project, 7-deazaguanine and several of its analogs were successfully prepared. It was discovered that 7-deazaguanine may easily be synthesized in two steps instead of three, resulting in a much better overall yield of the 7-deazapurine. Pentenyl ribosides were also successfully prepared for this study. Several different protecting groups were placed on the pentenyl ribosides to investigate their effectiveness in enhancing the stereoselectivity of the ribosylation reactions. All of the pentenyl ribosides and all of the 7-deazaguanine analogs were very stable and easy to manipulate in the laboratory.

Although pentenyl ribosides have been used in the past to prepare several nucleosides,[17] this study found that the method is not very useful in ribosylations containing pyrrolo[2,3-*d*]pyrimidines. The double bond of the pyrrolo ring is too reactive in the presence of I^+. Due to the vast number of side products formed in the coupling reactions, it is suspected that I^+ may initiate a free radical oxidation of the 7-deazapurine *in situ*. None of the 7-deazapurines could be recovered from the coupling attempts, although traces of the heterocyclic bases may have been lost during the work up of the reactions.

It should be noted that two successful ribosylations did occur using other nucleophiles. One experiment coupled pent-4-enyl 2',3',5'-tri-*O*-benzyl-D-*erythro*-pentofuranoside **39** to benzyl alcohol. The second experiment successfully coupled a

chlorinated purine analog to **39**. As expected, the benzyl group did not aid much in the stereoselectivity of the reaction and resulted in anomeric mixtures of the products.

The formation of 7-iodo-7-deazapurine **40** could not be confirmed upon addition of I^+. The compound was difficult to isolate by HPLC, but a compound with the expected UV spectrum and a bathochromic shift of 10 nm was present. A compound with the expected molecular weight was also detected by GC-MS, but the mass spectrum did not contain a strong M-I peak. This raises doubts about the presence of **40** in the reaction..

Hydrogenations of the reactive double bond in the 7-deazapurines were unsuccessful using both Pd/C and Raney nickel. This is probably due to the inherent stability of the ring system. Although an effective catalyst should be available for hydrogenation, it was beyond the scope of this project to investigate this possibility further. Once the double bond is removed, problems may exist in restoring the pyrrolo ring system without damaging the rest of the nucleoside.

It should be noted that we did find an online reference in the Glen Research archives discussing the instability of the phosphoramidite of 7-deazaguanosine in solutions containing iodine.[31] They report that using a 0.02 M iodine solution to oxidize oligonucleotides containing 7-deazaguanosine residues caused damage to the oligo-nucleotides. After HPLC analysis, the oligonucleotides contained major fault peaks corresponding to the location of the 7-deazaguanosine residues. They developed a new oxidizer for these reactions, and the results of these tests were reported in their online catalog. If any coupled 7-deazaguanosine products were formed in the ribosylation reactions, the coupled products would be damaged by the presence of iodine in the reaction.

CHAPTER IV

EXPERIMENTAL

General Methods

All reactions were carried out under an atmosphere of dry nitrogen and followed by thin-layer chromatography (TLC). Glassware was oven-dried before use and stored under nitrogen in a dessicator containing anhydrous calcium sulfate. Solvents were purified and dried by the methods described in *Purification of Laboratory Chemicals*[32] prior to use. Salts were dried in a vacuum oven (3 mmHg,100 °C) before use. All solid products were dried under vacuum over P_2O_5, and all oil products were placed under vacuum on a pump to remove traces of solvent before obtaining analytical data. TLC plates (Polygram® SIL G/UV_{254} from Alltech) were visualized with ammonium molybdate stain and/or UV light. Column chromatography was performed with silica gel (Selecto, 230-400 mesh) using 10 psi (flash column) or 40 psi (medium pressure column) air pressure. HPLC (C_{18} Microsorb™ column) was performed using a Waters 600 controller, a Waters 600E pump, a Waters 996 photodiode array detector, and Millenium 2010 chromatography software. Separation was accomplished with a 4.0 mL/min flow rate using a methanol/water solvent gradient. GC-MS was performed with a Hewlett Packard G1800A GCD system utilizing a 1.0 mL/min flow rate. ^1H and ^{13}C NMR data was collected at 300 MHz and 75 MHz, respectively, using a GEMINI 2000 High-Resolution NMR System (internal reference Me_4Si).

Preparation of Ethyl 2-Cyano-4,4-diethoxybutanoate[19](24). Ethyl cyanoacetate (500 mL, 4.70 mol) and bromoacetaldehyde diethylacetyl (142 mL, 0.940 mol) were combined in a 1-L round-bottomed flask fitted with a reflux condenser. Anhydrous potassium carbonate (130.6 g, 0.94 mol) and sodium iodide (9.33 g, 0.058 mol) were added to the mixture while stirring vigorously. The suspension was refluxed for 7 h and cooled to room temperature. The reaction was diluted with 100 mL of diethyl ether. Water (100 mL) was added to the suspension to dissolve the solids. After removing the organic layer, the aqueous phase was extracted twice with fresh diethyl ether (2 x 50 mL). The ether extracts were combined and washed with saturated sodium chloride and dried (Na_2SO_4). The filtered organic layer was concentrated using a rotary evaporator to give 400 mL of thick dark brown oil. The oil was purified by vacuum distillation to give 59 mL (0.250 mol, 27%) of **24** as a clear yellow oil (bp 115-133 °C, 3 mmHg). $R_f = 0.88$ (ethyl acetate); GC-MS: m/z = 229 ($C_{11}H_{19}NO_4$); ^{1}H NMR ($CDCl_3$) δ 4.66 (dd, J = 5.1, 6.3 Hz, 1 H), 4.23 (q, J = 7.1 Hz, 2 H), 3.66 (m, 3 H), 3.51 (m, 2 H), 2.24 (m, 2 H), 1.29 (t, J = 7.1 Hz, 3 H), 1.19 (t, J = 7.1 Hz, 3 H), 1.17 (t, J = 7.0 Hz, 3 H) ; ^{13}C NMR ($CDCl_3$) δ 166.0, 116.4, 100.0, 62.8, 62.6 (2 C), 33.5, 33.4, 15.1, 15.0, 13.8.

Preparation of 2,4-Diamino-5-(2,2-diethoxyethyl)pyrimidin-6-one[19] (25). Sodium ethoxide was first prepared by refluxing 14.1 g (610 mmol) of sodium metal with 300 mL of anhydrous ethanol in a 500-mL round-bottomed flask. Guanidine was prepared by stirring dried guanidine•HCl (23.9 g, 250 mmol) with 150 mL of the freshly prepared ethanolic sodium ethoxide (2 M, 250 mmol) until dissolved. The guanidine solution was filtered through a fritted glass filter under N_2 into the 500-mL round-bottomed flask containing the remaining 150 mL of ethanolic sodium ethoxide. Next, **24**

(59 mL, 59.1 g, 250 mmol) was added to the guanidine solution dropwise with stirring. The reaction was refluxed for 2 h and cooled to room temperature. After cooling, the mixture was transferred to a 1-L round-bottomed flask and distilled under vacuum to remove the ethanol. As the volume decreased, 20 mL of water was added to reduce the violent bumping that occurred. The sticky residue was taken up in an additional 80 mL of water and washed with fresh ether (3 x 100 mL). The aqueous layer was acidified with acetic acid (14.3 mL, 250 mmol) to a pH of 4-5. A precipitate quickly formed. The mixture was cooled in an ice bath and vacuum filtered. The resulting yellow solid was recrystallized from water to yield 27.5 g (110 mmol, 46%) of **25** as a fine, white, crystalline solid, mp 183.5-184.0 °C. R_f = 0.26 (ethyl acetate); GC-MS: *m/z* = 242 ($C_{10}H_{18}N_4O_3$); ^{1}H NMR (d_6-DMSO) δ 9.82 (bs, 1 H), 5.97 (bs , 2 H), 5.59 (bs , 2 H), 4.42 (t, *J* = 5.5 Hz, 1 H), 3.56 (q, *J* = 7.0 Hz, 2 H), 3.37 (q, *J* = 7.0 Hz, 2 H), 2.41 (d, *J* = 5.5 Hz, 2 H), 1.06 (t, *J* = 7.0 Hz, 6 H); ^{13}C NMR (d_6-DMSO) δ 162.9, 155.0, 102.9, 83.4, 61.4, 28.7, 15.3.

Preparation of 2-Aminopyrrolo[2,3-*d*]pyrimidin-4-one (7-deazaguanine)[19] (**26**). To a 1-L round-bottomed flask containing 27.0 g (110 mmol) of **25** was added 558 mL (2 M, 165 mmol) of hydrochloric acid. After stirring at room temperature for 4.5 h, the solution was neutralized with 7.30 mL (165 mmol) of concentrated ammonium hydroxide. White solids precipitated out of the solution, and the precipitate was collected by vacuum filtration. Recrystallization of the precipitate from water yielded 14.5 g (100 mmol, 93%) of **26** as a salmon-colored crystalline solid, mp 294.5-296.0 °C (dec). R_f = 0.02 (90:10, chloroform: ethanol); GC-MS: *m/z* = 150 ($C_6H_6N_4O$); FAB: M^{+1} = 151; ^{1}H NMR (d_6-DMSO) δ 10.98 (bs, 1 H), 10.25 (bs, 1 H), 6.60 (dd, *J* = 3.4, 2.2 Hz, 1 H), 6.18

(dd, J = 3.3, 2.2 Hz, 1 H), 6.07 (bs, 2 H); ^{13}C NMR (d_6-DMSO) δ 158.9, 152.3, 151.2, 116.7, 101.6, 99.9.

Preparation of Pent-4-enyl D-*erythro*-Pentofuranoside[17] (28). In a 250-mL round-bottomed flask were placed 6.64 g (44.2 mmol) of D-ribose and 50 mL (43.4 g, 504 mmol) of 4-penten-1-ol. The mixture was stirred, and a solution of 453 mg (1.95 mmol, 0.08 mmol) of (±)-10-camphorsulfonic acid dissolved in an additional 2 mL of the pentenyl alcohol was added to the sugar solution. The reaction was allowed to stir at room temperature for 48 h, until the cloudy suspension became a clear solution. Once the solution cleared, 3.70 g (13.4 mmol) of silver carbonate was added, and the resulting suspension was allowed to stir for an additional 0.75 h. The solids were filtered out of the solution using a fritted glass filter. The filtrate was distilled under vaccuum to give11.4 g of an oil with a slight purple tint. The oil was purified by medium pressure chromatography (99:1 to 90:10, chloroform: ethanol gradient) to yield 6.23 g (28.5 mmol, 65%, β) of **28a**, 1.83 g (8.38 mmol, 19%, α) of **28b**, and 0.88 g (4.03 mmol, 9%) of mixed anomers as clear oils, giving an overall yield of 8.94 g (40.9 mmol, 93%) of **28**. R_f (β) = 0.21; R_f (α) = 0.27 (90:10, chloroform: ethanol). ^{1}H NMR ($CDCl_3$) δ 5.75 (ddt, J = 17.9, 9.8, 7.2 Hz, 1 H), 5.0 (dq, J =17.9, 2.7 Hz, 2 H), 4.96 (dq, J = 9.8, 2.7 Hz, 1 H), 4.28 (t, J = 5.4 Hz, 1 H), 4.03 (dt, J = 5.4, 5.4 Hz, 2 H), 3.70 (m, 3 H), 3.43 (dt, J = 9.8, 6.2 Hz, 2 H), 3.35 (bs, 3 H), 2.08 (m, 2 H), 1.69 (m, 2 H).

Preparation of Pent-4-enyl 2',3',5'-Tri-*O*-benzoyl-β-D-*erythro*-pentofuranoside [17] (29). A 50-mL round-bottomed flask was charged with **28a** (1.79 g, 8.2 mmol) and pyridine (20 mL, 248 mmol). The solution was stirred, and benzoyl chloride (5.07, 43.5 mmol, excess) was added next dropwise. After 1.5 h, the apricot-colored solution

was distilled under vacuum to remove the pyridine. The residue was redissolved in 200 mL of 50:50 ethyl acetate:water. After removing the aqueous phase, the organic layer was washed with 100 mL of 1 M sulfuric acid, 100 mL of 1 M sodium bicarbonate, 50 mL of saturated sodium chloride, and dried (Na_2SO_4). After filtering, the organic solution was distilled under vacuum to give a light amber colored oil. The crude oil was purified by flash chromatography (95:5, hexane: ethyl acetate) to yield 3.48 g (6.56 mmol, 80%) of **29** as a clear oil. Rf = 0.08 (97:3, hexane: ethyl acetate). ^{1}H NMR ($CDCl_3$) δ 8.16, 8.15, 8.14, 8.12, 8.07, 8.03 (6 x d, 6 H), 7.53 (m, 9 H), 5.88 (dd, J = 6.9, 4.9, 1 H), 5.75 (ddt, J = 17.2, 9.8, 7.2 Hz, 1 H), 5.68 (d, J = 4.3 Hz, 1 H), 5.25 (s, 1 H), 4.98 (m, 2 H), 4.71 (m, 2 H), 4.56 (dd, J = 12.8, 5.9 Hz, 1 H), 3.78 (dt, J = 9.5, 6.6 Hz, 1 H), 3.45 (dt, J = 9.5, 6.6 Hz, 1 H), 2.07 (m, 2 H), 1.63 (m, 2 H).

Attempted coupling of 26 and 29 using NIS and TfOH[17] . To a 50-mL round-bottomed flask containing 16.5 mL of acetonitrile over activated 4 Å molecular sieves were added 50.0 mg (0.33 mmol) of **26** and 263 mg (0.49 mmol) of **29**. N-iodosuccinimide (100 mg, 0.50 mmol) was added, and the light brown suspension was stirred vigorously. After 5 minutes, 44.5 μL (0.50 mmol) of triflic acid was added, and the suspension immediately turned dark green. After 40 min, the reaction was quenched with 16.5 mL (0.50 mmol) of 0.03 M pyridine in dichloromethane. The suspension was distilled under vacuum to give a dark green residue. The residue was taken up in 50 mL of diethyl ether and washed with 100 mL of 1 M sulfuric acid, 100 mL of 1 M sodium bicarbonate, 50 mL of saturated sodium chloride, and dried (Na_2SO_4). The organic layer was distilled under vacuum to give a thick oil. The oil was purified by flash chromatography (99:1 to 97:3, chloroform:ethanol gradient). After analysis of the

separated fractions, no coupled products could be detected by FAB, GC-MS, or ^{1}H NMR. The only compounds identified were **29** and **30**.

Preparation of 2-Acetamido-7-acetylpyrrolo[2,3-*d*]pyrimidin-4-one[21] (31). A 100-mL round-bottomed flask fitted with a reflux condensor was charged with 2.01 g (10 mmol) of **26** and 30 mL (380 mmol) of pyridine. Acetic anhydride (7.0 mL, 74 mmol) was added dropwise, followed by a catalytic amount of DMAP (81.2 mg, 0.67 mmol). The mixture was refluxed for 5.5 h and cooled to room temperature. The solution was transferred to a 250 mL round-bottomed flask and distilled under vacuum to remove the pyridine. Water (50 mL) was added to the residue, and the resulting suspension was allowed to stir for 0.5 h. The suspension was cooled in an ice bath, and the solids were collected by vacuum filtration. The crude solid (2.54 g, 10.8 mmol) was recrystallized from ethanol to yield 1.86 g (7.90 mmol, 81%) of **31** as light biege needles, mp 279-281 °C (dec.). R_f = 0.39 (90:10 chloroform: ethanol); ^{1}H NMR (d_6-DMSO) δ 12.01 (bs, 1 H), 11.49 (bs, 1 H), 7.48 (d, J = 4.4 Hz, 1 H), 6.62 (d, J = 4.4 Hz, 1 H), 2.85 (s, 3 H), 2.21 (s, 3 H); ^{13}C NMR (d_6-DMSO) δ 169.6, 164.6, 152.4, 144.3, 143.4, 115.4, 103.1, 101.3, 21.1, 19.7.

Preparation of 2-Acetamidopyrrolo[2,3-*d*]pyrimidin-4-one[20] (32). To a stirring suspension of 2.00g (8.5 mmol) of **31** in 95 mL of methanol was added 1.24 mL (18.8 mmol) concentrated ammonium hydroxide. Cold water was introduced into the reaction after 0.5 h, and the suspended solid was collected in a fritted glass filter. The resulting cake was thoroughly washed with additional cold water and dried for 12 h under vaccuum over P_2O_5. Recrystallization of the crude solid proved difficult, but proton NMR of the crude solid indicated that recrystallization was not required. The product, **32**, was

obtained as 1.43 g (7.44 mmol, 88%) of light biege powder. R_f = 0.14 (90:10, chloroform: ethanol); ^{1}H NMR (d_6-DMSO) δ 11.83 (bs, 2 H), 11.49 (bs, 1 H), 6.96 (dd, J =2.3, 3.4 Hz, 1 H), 6.39 (dd, J =2.3, 3.4 Hz, 1 H), 2.85 (s, 3 H); ^{13}C NMR (d_6-DMSO) δ 169.2, 152.9, 143.9, 142.1, 115.6, 99.6, 98.1, 19.5.

Attempted Coupling of 29 and 32 using NIS and TfO-TMS.[18] In a 25-mL round-bottomed flask were placed 142 mg (0.27 mmol) of **32** and 60.8 mg (0.27 mmol) of **29** in 4 mL (4.8 mmol) of acetonitrile. The suspension was stirred, and 60.8 mg (0.27 mmol) of *N*-iodosuccinimide was added. The suspension turned dark green, and after adding TfO-TMS, the suspension turned black. The reaction was quenched after 0.5 h with 1.5 mL of 10% $Na_2S_2O_3$ solution and 1.5 mL of a saturated $NaHCO_3$ solution. The reaction was placed in a separatory funnel and extracted with diethyl ether (2 x 45 mL). The combined ether layers were dried and distilled under vacuum to give a yellow residue. No coupled products could be detected by TLC, HPLC or ^{1}H NMR. The only identifiable compounds were **29** and **30**.

Preparation of 2-Amino-4-chloropyrrolo[2,3-*d*]pyrimidine[4] **(10)**. A 100-mL round-bottomed flask fitted with a reflux condensor was charged with **26** (2.82 g, 18.8 mmol) and $POCl_3$ (46.0 g, 28.0 mL, 301 mmol). Dimethylaniline (548 μL, 526 mg, 4.33 mmol) was added to the mixture dropwise, with stirring. The reaction was refluxed for 6 h, then cooled to room temperature. The cooled mixture was distilled under vacuum to remove the $POCl_3$. The resulting yellow oil was transferred to a 1-L round-bottomed flask, and 500 g of crushed ice was added with vigorous mixing. A precipitate formed immediately. The suspension was heated to reflux, cooled in an ice bath, and filtered to remove any insoluble impurities. The aqueous filtrate was adjusted to a pH ≥ 2 with

concentrated ammonium hydroxide, and a new white precipitate immediately formed. The suspension was further cooled in an ice bath, and the solid was collected in a fritted glass filter. The solid was dried for 12 h under vacuum over P_2O_5, and1.73 g (10.3 mmol, 55%) of **10** was obtained as a green powder. GC-MS: *m/z* = 168 ($C_6H_5ClN_4$); R_f = 0.20 (90:10, chloroform: ethanol). ^{1}H NMR (d_6-DMSO) δ 11.47 (bs, 1 H), 7.06 (dd, *J* = 3.5, 2.2 Hz, 1 H), 6.49 (bs, 2 H), 6.23 (dd, *J* =3.6, 1.8 Hz, 1 H); ^{13}C NMR (d_6-DMSO) δ 159.3, 154.6, 150.9, 123.2, 108.6, 98.7.

Preparation of 2-Amino-4-methoxypyrrolo[2,3-*d*]pyrimidine[4] (**33**). Sodium methoxide was prepared by stirring 4 mL (98 mmol) of anhydrous methanol and 46.0 mg (2 mmol) of sodium metal in a 25-mL round-bottomed flask until all of the sodium had dissolved. To this solution was added 101 mg (0.60 mmol) of **10**. The mixture was refluxed for 15 h, and the reaction was followed by GC-MS. Once the peak for the starting material had disappeared, the solution was cooled to room temperature. After cooling, 15 mL of water was added, and the resulting solution was acidified to a pH of 4-5 with concentrated hydrochloric acid. The acidified aqueous solution was extracted with ethyl acetate (3 x 20 mL), and the combined ethyl acetate extracts were washed with saturated sodium chloride and dried (Na_2SO_4). The filtered organic layer was distilled under vacuum to give an off-white residue. The residue was recrystallized from toluene to yield 68.4 mg (0.42 mmol, 69%) of **33** as an off-white powder, mp 195-197 °C. GC-MS: *m/z* = 164 ($C_7H_8N_4O$); ^{1}H NMR (d_6-DMSO) δ 11.475 (bs, 1 H), 6.82 (dd, *J* = 3.4 Hz, 2.2, 1 H), 6.18 (dd, *J* = 3.5, 1.9 Hz, 1 H), 5.98 (bs, 2 H), 3.87 (s, 3 H); ^{13}C NMR (d_6-DMSO) δ 158.8, 155.4, 151.0, 115.2, 93.9, 92.8, 48.4.

Attempted Coupling of 33 and 34 using NIS and TfO-TMS.[18] A 25-ml round-bottomed flask was charged with 70.7 mg (0.21 mmol) of **34**, 33.7 mg (0.21 mmol) of **33**, and 2 mL of acetonitrile. The flask was covered in aluminum foil to protect the reaction from light. After thoroughly stirring the suspension, 46.1 mg (0.21 mmol) of NIS was added. The reaction turned dark brown. After 1 min, 33 μL (0.21 mmol) of TfO-TMS was added. The reaction was quenched after 20 h with 3.0 mL of a 10% $Na_2S_2O_3$ solution and 3.0 mL of a saturated $NaHCO_3$ solution. The organics were extracted with ethyl acetate (3 x 20 mL) and dried (Na_2SO_4). The filtered organic layer was distilled under vacuum to give 73.2 mg of a dark brown residue. The residue was mostly composed of **34** and **35**.

Preparation of pent-4-enyl 2',3',5'-Tri-*O*-benzyl-β-D-*erythro*-pentofuranoside[25] **(36)**. An mixture of **28a** and **28b** (1.88 g, 8.70 mmol) was placed in a 100-mL round-bottomed flask containing 12 mL of tetrahydrofuran (THF). Powdered potassium hydroxide (6.53 g, 116 mmol) was added with stirring. After thorough mixing, 9.22 mL (78.0 mmol) of benzyl chloride was added dropwise, and the reaction was heated to reflux. The reaction was cooled after 24 h and filtered through a fritted glass filter to remove solid impurities. The resulting cake was rinsed thoroughly with fresh tetrahydrofuran, and the combined filtrates were distilled under vacuum to yield a pale yellow oil. The crude oil was purified by medium-pressure chromatography (95:5 to 90:10, hexane: ethyl acetate gradient) to yield 2.73 g (5.58 mmol, 64%) of **36a**, 0.330 g (0.67 mmol, 8%) of **36b**, and 0.354 g (0.72 mmol, 8%) of mixed anomers as clear oils. The overall yield was 3.410 g (6.97 mmol, 80%) of mixed products. R_f (β) = 0.48; R_f (α) = 0.36 (80:20, hexane: ethyl acetate). ^{1}H NMR ($CDCl_3$) δ 7.30(m, 15 H), 5.80 (m, 9

H), 5.88 (ddt, J = 17.2, 9.8, 7.2 Hz, 1 H), 4.98 (m, 2 H), 4.65 (m, 3 H), 4.56 (m, 3 H), 4.35 (m, 1 H) 4.22 (m, 1 H), 3.79 (m, 2 H), 3.55 (m, 1 H), 3.4 (m, 1 H), 2.02 (m, 2 H), 1.59 (m, 2 H).

Preparation of Silver Di-sym-collidine Perchlorate (AgDPC)[26] (37). To a stirring aqueous solution of silver nitrate (4.49 g, 26.4 mmol) and sodium perchlorate (5.49 g, 44.8 mmol) was added 10 mL of 2,4,6-collidine (9.15 g, 75.5 mmol). A white precipitate formed, and the reaction was allowed to stir for 0.5 h. The white precipitate was collected in a fritted glass filter, and the resulting cake was thoroughly washed with cold water (6 x 25 mL). The cake was further washed with cold ethanol (2 x 35 mL) to remove any excess collidine and allowed to air dry. The solid was dried for 12 h under vacuum over P_2O_5, and 11.6 g (25.8 mmol, 98%) of **37** was obtained as a white powder.

Preparation of IDPC[26] (38). A 500-mL round-bottomed flask was charged with 10.7 mg (23.8 mmol) of **37** and 1.51 mL (11.9 mmol) 2,4,6-collidine in 60 mL of chloroform. After thoroughly stirring the mixture, 5.00 g (19.8 mmol) of iodine crystals was added. The suspension turned bright yellow. The reaction was allowed to stir under anhydrous conditions for 0.15 h, then the yellow precipitate was filtered over celite in a fritted glass filter. The deep maroon filtrate was sealed and placed in a refrigerator for 12 h. Course white crystals formed in the filtrate. The crystals were collected in a fritted glass filter and dried for 12 h under vacuum over P_2O_5. The product, **37**, was obtained as 4.72 g (10.1 mmol, 38%) of coarse white crystals.

Attempted Coupling of 10 and 36 using IDPC.[18] A 10-ml round-bottomed flask was charged with 31.0 mg (0.06 mmol) of **36**, 20.2 mg (0.12 mmol) of **10**, and 1 mL of acetonitrile. After thoroughly stirring the suspension, 32.4 mg (0.07 mmol) of IDPC was

added was added. The reaction turned dark green. The reaction was quenched after 0.5 h with 1.0 mL of a 10% $Na_2S_2O_3$ solution and 1.0 mL of a saturated $NaHCO_3$ solution. The organics were extracted with ethyl acetate (3 x 10 mL) and dried (Na_2SO_4). The filtered organic layer was distilled under vacuum to give a dark green residue. The residue was mostly composed of the hydrolyzed sugar, **37**.

Attempted Halogenation of 2-Amino-4-methoxypyrrolo[2,3-*d*]pyrimidine. A 10-ml round-bottomed flask was charged with 52.4 mg (0.33 mmol) of **33**, 109 mg (0.48 mmol) of NIS, and 4 mL of tetrahydrofuran. The reaction turned dark green upon stirring. After 4 h, the reaction was quenched with 2.0 mL of a 10% $Na_2S_2O_3$ solution and 2.0 mL of a saturated $NaHCO_3$ solution. The mixture was distilled under vacuum to remove the tetrahydrofuran, then the remaining solution was extracted with ethyl acetate (3 x 10 mL) and dried (Na_2SO_4). The filtered organic layer was distilled under vacuum to give a brown residue with black solids. The residue was purified with a silca gel/celite mini column using ethyl acetate. The resulting yellow solution was distilled under vacuum to give a green residue. GC-MS: m/z = 290, but no M-I was present; ^{1}H NMR (d_6-DMSO): 2:1 ratio of **33**:unknown aromatic peaks.

Preparation of 1',2',3',5'-tetra-*O*-benzyl-β-D-*erythro*-pentofuranoside using IDPC [18] (**40**). To a 15-mL round-bottomed flask containing 71.8 mg (0.147 mmol) of **36** was added 1.5 mL of methylene chloride and 15 μL (0.147 mmol) benzyl alcohol. The reaction vial was stirred thoroughly, and 103 mg (0.220 mmol) of IDPC was added. The mixture became a yellow solution upon stirring. After 0.45 h, the reaction was quenched with 3.0 mL of a 10% $Na_2S_2O_3$ solution. An additional 10 mL of methylene chloride was added, and the reaction was filtered through a pipette filled with celite to remove any

isoluble material. The organic layer was separated and washed with 3 mL of a saturated $NaHCO_3$ solution, 2 mL of saturated sodium chloride and dried (Na_2SO_4). After filtering, the organic layer was distilled under vacuum to give an oil. The crude oil was purified by flash chromatography (97:3 to 95:5, hexane:ethyl acetate gradient) to yield 17 mg (0.032 mmol, 22%) of **40a** and 4.7 mg (0.009 mmol, 6%) of **40b** as clear oils. R_f = 0.43, 0.33 (80:20, hexane:ethyl acetate). ^1H NMR ($CDCl_3$) δ 7.25 (m, 20 H), 4.97 (m, 1 H), 4.71 (m, 1 H), 4.59 (m, 2 H), 4.45 (m, 1 H), 4.28 (m, 1 H), 3.85 (2 x d, J = 4.1 Hz, 2 H) 3.78 (2 x d, J = 4.1 Hz, 2 H), 3.45 (2 x d, J = 4.1 Hz, 2 H), 3.35 (2 x d, J = 4.1 Hz, 2 H).

Preparation of 2,6-dichloro-9-(D-ribofuranosyl)purine (42) and 2,6-dichloro-7-(D-ribofuranosyl)purine (43) [27] [Coupling reaction using *in situ* IDPC] To a 25-mL round-bottomed flask containing 2 mL anhydrous acetonitrile over 3 Å molecular sieves was added 44.9 mg (0.10 mmol) of **37** and 25.3 mg (0.10 mmol) of powdered iodine. Upon mixing, the reaction turned into an orange solution with a yellow precipitate. After stirring for 1.5 h, 18.9 mg (0.10 mmol) of dichloropurine **41** was added, and no color change was noted. The suspension was allowed to stir for 5 min, then 48.8 mg (0.10 mmol) of **36** was introduced into the reaction. The color remained constant throughout the additions. After 4 h, the reaction was quenched with 1.0 mL of a 10% $Na_2S_2O_3$ solution and 1.0 mL of a saturated $NaHCO_3$ solution. The organics were extracted with ethyl acetate (3 x 10 mL) and dried (Na_2SO_4). The filtered organic layer was distilled under vacuum to give 49.5 mg of clear oil. The oil was purified by flash chromatography (95:5 to 80:20, hexane:ethyl acetate gradient) to give six oil products. The compounds were identified as 19 mg of **36**, 1.5 mg of **39**, and 10.7 mg of a mixture of **43** and **44**.

FAB analysis of the fractions containing **43** and **44** showed an M^{+1} of 591, matching the expected molecular weight of the coupled product.

Attempted hydrogenation of 2-Amino-4-methoxypyrrolo[2,3-*d*]pyrimidine using Pd/C.[28] To a 25-mL beaker was added 279 mg (1.45 mmol) of **32** and 30 mg (10% of **32** by weight) of 10% Pd/C in 10 mL of methanol. The beaker was placed in a pressure reactor, and the vessel was thoroughly degassed. The vessel was flooded with hydrogen gas and degassed for a total of three times. Hydrogen gas was bubbled through the reactor for 4 min, then the reaction vessel was placed under 40 psi (2.72 atm) hydrogen gas pressure for 8 h. No reaction could be detected, so the pressure was increased to 60 psi (4.0 atm). The reaction was allowed to stir under pressure for 4 d. No reaction took place. GC-MS and ^{1}H NMR showed only starting materials were present.

Attempted hydrogenation of 7-deazaguanine using Raney Nickel.[29, 30] To a 25-mL beaker was added 227 mg (1.50 mmol) of **26** and 30 μL (45 mg, 20%) of Raney nickel in 5 mL of ethanol. The beaker was placed in a pressure reactor, and the vessel was thoroughly degassed. The sealed reactor was flooded with hydrogen gas and degassed three times. Hydrogen gas was bubbled through the reactor for 10 min, then the reaction vessel was placed under 60 psi (4.0 atm) hydrogen gas pressure. The reaction was allowed to stir under pressure for 7 d. No reaction took place. GC-MS and ^{1}H NMR showed only starting materials were present.

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