THE SYNTHESIS OF CERTAIN CARBOCYCLIC NUCLEOSIDE ANALOGS AS ANTIVIRAL AGENTS (U)
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THE SYNTHESIS OF CERTAIN CARBOCYCLIC NUCLEOSIDE ANALOGS AS ANTIVIRAL AGENTS

FINAL REPORT

John A. Secrist III

DECEMBER 1985

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-84-C-4135

Southern Research Institute
Birmingham, Alabama 35255-5305
Project No. 5647-IV

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Antiviral; synthesis; nucleoside; carbocyclic 3-deazaadenosine

The goal of this project was the synthesis of new candidate antiviral drugs for evaluation in the U. S. Army Medical Research Institute of Infectious Diseases virus panel. Specifically, compounds were designed to resemble carbocyclic 3-deazaadenosine, our lead compound, in having the following characteristics: 1) a resemblance to adenine nucleosides, 2) little or no substrate activity for adenosine deaminase, and 3) little or no substrate activity for any nucleoside kinases. The synthetic schemes employed involved the building up of the carbocyclic nucleosides from a cyclopentane precursor by condensation with an appro-
appropriate nitrogen heterocycle. Manipulations were then carried out at the 5'-carbon of the carbocyclic nucleosides. Several compounds were prepared and submitted for testing, and chemistry leading to a larger series was developed, such that these other compounds can be prepared in the near future.
SUMMARY

The goal of this project was the synthesis of new candidate antiviral drugs for evaluation in the U. S. Army Medical Research Institute of Infectious Diseases virus panel. Specifically, compounds were designed to resemble carbocyclic 3-deazaadenosine, our lead compound, in having the following characteristics: 1) a resemblance to adenine nucleosides, 2) little or no substrate activity for adenosine deaminase, and 3) little or no substrate activity for any nucleoside kinases. The synthetic schemes employed involved the building up of the carbocyclic nucleosides from a cyclopentane precursor by condensation with an appropriate nitrogen heterocycle. Manipulations were then carried at the 5'-carbon of the carbocyclic nucleosides. Several compounds were prepared and submitted for testing, and chemistry leading to a larger series was developed, such that these other compounds can be prepared in the near future.
FOREWORD

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.
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A. Introduction

This report summarizes the research carried out under Contract No. DAMD17-84-C-4135, 15 September 1984 - 14 December 1985.

B. Background

Carbocyclic nucleosides, that is, nucleosides that have the ribofuranosyl ring oxygen replaced by a -CH₂- unit, have been known for some years.¹⁻³ Although they resemble normal nucleosides, the glycosidic bond is no longer at the aldehyde oxidation level. One consequence of this property is that carbocyclic nucleosides are not susceptible to cleavage by phosphorylases. Conformational and electronic differences also result from this subtle structural change. From among the various compounds prepared in this class, some have been found to have interesting biological properties.

Several carbocyclic purine analogues have antiviral activity in vitro. Among those with activity against herpes simplex type 1 (HSV 1) and vaccinia viruses are the carbocyclic analogues of inosine (2a), 8-azainosine (2b), O⁶-methylinosine (1a), 6-chloropurine ribonucleoside (1b), and 6-(methylthio)purine ribonucleoside (1c). All of these compounds are either not toxic or only moderately toxic to H.Ep.-2 cells. Compounds 1a, 1c, and 2a are as active as ara-A against the named viruses.⁴ Carbocyclic 2,6-diaminopurine ribonucleoside (1d) also has activity against HSV-1 in vitro, but not in vivo.⁵a A series of 2'-deoxyribofuranosides of 2-amino-6-substituted-purines and 8-azapurines also have activity against HSV-1 and HSV-2.⁶⁻¹⁰

The carbocyclic analogue of ara-A (3) appears to show considerable promise as an antiviral agent. It has in vivo activity against HSV-1 encephalitis in mice and against HSV-2 genital infections in guinea pigs.⁶ This compound is currently under active investigation.

A number of carbocyclic pyrimidine nucleoside analogues are highly active antiviral agents in vitro. Among the best are 5,⁷ 4a,⁶ and carbocyclic 5-ido-2'-deoxyuridine (4b).⁶ Compound 5 is highly active against human influenza virus in vitro, but as yet has shown no in vivo activity. Carbocyclic 5-ido-2'-deoxyuridine (4b) has excellent in vitro activity against HSV-1, strain 377, and HSV-2, strain MS.⁶ It also has in vivo activity against HSV-1 in mice, as does carbocyclic thymidine (4a).⁸ Carbocyclic 5-ido-2'-deoxyuridine (4b) is superior to 5-ido-2'-deoxyuridine against HSV-1.⁸

Recently, another carbocyclic nucleoside, carbocyclic 3-deazaadenosine (6b), has been shown to have promise as an antiviral agent.⁹,¹⁰ This compound was designed to be
a) $X = \text{CH}$, $R = \text{OCH}_3$, $R_1 = \text{H}$
b) $X = \text{CH}$, $R = \text{Cl}$, $R_1 = \text{H}$
c) $X = \text{CH}$, $R = \text{SCH}_3$, $R_1 = \text{H}$
d) $X = \text{CH}$, $R = R_1 = \text{NH}_2$

a) $R = \text{CH}_3$
b) $R = \text{I}$

a) $X = \text{O}$
b) $X = \text{CH}_2$
a specific inhibitor of S-adenosyl-L-homocysteinase. It has been established that 3-deazaadenosine (6a) is a good inhibitor of the enzyme and that it has in vitro antiviral activity against certain RNA viruses, including vesicular stomatitis, Rous sarcoma, Sindbis, and Newcastle disease viruses. 3-Deazaadenosine is not a potent antiviral agent, however, nor does it have an attractive therapeutic index. Combining the features of C-Ado and 6a suggested the synthesis of 6b.

Compound 6b exhibited in vitro activity against vaccinia, vesicular stomatitis, Sindbis, measles, Coxsackie type B4, parainfluenza type 3, and reo type 1 viruses. With all of these viruses, no cytotoxicity was seen at the highest levels examined (cell lines employed were primary rabbit kidney cells, Vero cells, HeLa cells, L-929 cells, and human embryonic skin-muscle fibroblasts), which were 20-200 fold higher than the minimum inhibitory concentration (0.2-20 μg/mL), with most ~1 μg/mL. Compound 6b was about 100 times more potent than other established broad-spectrum antiviral agents such as ribavirin and (S)-9-(2,3-dihydroxypropyl)adenine against vesicular stomatitis, parainfluenza, measles, and reo viruses. In vivo activity has also been seen with 6b. A single dose of 6b (20, 100, or 500 μg per mouse) administered one hour after infection protected newborn mice against a lethal infection of vesicular stomatitis virus.

The suggested mechanism of action of 6b is the inhibition of a key methylation reaction by feedback inhibition resulting from the inhibition of S-adenosyl-L-homocysteinase. Compound 6b is an excellent competitive inhibitor of the enzyme from several sources. It is not a substrate for adenosine deaminase, and is not phosphorylated by L1210 leukemia cells. Hence a mechanism of action at the nucleoside level is reasonable. Increases in the levels of AdoMet were seen in both normal rat kidney cells (NRK 153 C1 7) and mouse L cells after administration of 6b.

Although a mechanism involving the inhibition of S-adenosyl-L-homocysteinase has been suggested for (6b), no proof yet exists. Other mechanisms of action, such as the inhibition of an enzyme along the polyamine biosynthetic pathway, are also logical. In fact, S-adenosylmethionine decarboxylase is inhibited by 6b as well as by 5'-deoxy-2-fluoro-5'-iodoadenosine (7). Compound 7, of course, like 6b, is neither deaminated nor phosphorylated. It is, however, cleaved by MTA phosphorylase to the highly toxic 2-fluoroadenine, and therefore we have not seriously considered it as a candidate antiviral agent. The corresponding carbo cyclic analogue would not be a substrate for the phosphorylase, and is a strong candidate for synthesis and testing as an antiviral agent. This type of compound is included in our list of target structures.
Target Structures

Based upon the background presented above, especially the exciting antiviral activity of carbocyclic 3-deazaadenosine (6b), we have undertaken to synthesize a series of carbocyclic nucleosides related to 6b and based upon the features of that compound that appear important for antiviral activity. Those features are 1) a resemblance to adenine nucleosides, 2) little or no substrate activity for adenosine deaminase, and 3) little or no conversion to the 5'-phosphate. The target compounds, all of which meet the above criteria, are listed in Table 1.

The bases present in structures 8-11 were chosen because in the corresponding standard ribonucleosides (3-deazaadenosine, 7-deazaadenosine, 1-deazaadenosine, and the 2-haloadenosines) they are highly resistant to deamination by adenosine deaminase. The 5'-substituents chosen, included in the R groups, will prevent any phosphorylation of the carbocyclic nucleoside. The only possible exception might be the 5'-amino group. It is known that the 5'-amino-5'-deoxy pyrimidine nucleosides that are active antiviral agents (against herpes simplex) are phosphorylated by a virally induced kinase. Normal cells, however, do not phosphorylate these compounds, and in our case we do not expect significant phosphorylation of the 5'-amino group. The incorporation of the isobutylthio group is based upon the antiviral activity of 5'-deoxy-5'-(isobutylthio)-3-deazaadenosine. Similarly, the 5'-amide and ester are included because not only will they prevent phosphorylation, but also because we have seen some antiviral activity with the 5'-amide of carbocyclic adenosine.

The three individual structures 12-14 all have 3-deazaadenine as the base. Again, all three compounds should meet the criteria listed above. The 2'-deoxy carbocycle in 12 is included based upon the good inhibition of S-adenosyl-L-homocysteinase by 2'-deoxyadenosine. Compounds 13 and 14 are included as new variations fitting the proposed rationale. Compound 14 is the only non-carbocyclic nucleoside on the target structure list. These target structures represent about 2-4 years of research, depending upon how the synthetic routes proceed.

C. Chemistry

From the large body of proposed target structures, we chose to head first toward those that were based directly upon carbocyclic 3-deazaadenosine (6b). For virtually all of our target structures, then, we needed 6b as the starting material. A considerable portion of our time was taken up in the synthesis of this material. The two building blocks
Table 1

a) CH₂Cl
b) CH₂Br
c) CH₂I
d) CH₃
e) CH₂SR' (R' = CH₂CH(CH₃)₂, (CH₂)₃CH₃, CH₃, etc.)
Scheme 1

\[
\begin{align*}
17 & \xrightarrow{\Delta} 18 \\
SO_2Na & \xrightarrow{ClCN} SO_2CN \text{ (19)} \\
1) \text{Aq. HCl} & \xrightarrow{2) CH_3OH, H^+} 22 \xrightarrow{HCl} 23 \\
& \xrightarrow{C_2H_5N} 24 \xrightarrow{NaBH_4} 25 \\
& \xrightarrow{Ba(OH)_2} 15
\end{align*}
\]

Scheme 2

\[
\begin{align*}
26 & \xrightarrow{\Delta} 27 + 28 \\
& \xrightarrow{HNO_3} 29 \xrightarrow{POCl_3} 16
\end{align*}
\]
for \(6b\) are the cyclopentylamine \(15\) and the pyridine derivative \(16\). The amine \(15\) is prepared as shown in Scheme 1. The pyridine derivative \(16\) is synthesized as shown in Scheme 2.

The preparation of \(15\)\(^{20,21}\) shown in Scheme 1 is considerably streamlined from the original synthesis,\(^{22}\) but is still somewhat involved, and requires considerable care at several stages. The cyclopentadiene \((18)\) and tosyl cyanide \((20)\) are prepared fresh and used immediately, and on large-scale, extreme care needs to be taken in the conversion of the initially formed adduct between \(18\) and \(20\) to lactam \(21\). Oxidation of the double bond of \(21\) with \(\text{KMnO}_4\) gives exclusively the desired cis glycol \(22\) in about 45% yield. The lactam is then opened with aqueous HCl and the methyl ester \(23\) formed with methanolic hydrogen chloride (57% for the two steps). After acetylation to produce \(24\), the methyl ester is reduced with sodium borohydride in the presence of calcium chloride. This procedure also reduces the acetyl groups, giving \(25\). Saponification of the amide is carried out with barium hydroxide to form the desired amine \(15\).

The synthesis of \(16\) is considerably shorter, starting from 4-nitropyridine N-oxide \((26)\). Heating \(26\) in acetic anhydride results in the formation of \(27\) and \(28\), from which \(27\) can be obtained pure if desired. Treatment of \(28\) (or a mixture of \(27\) and \(28\)) with nitric acid converts it to \(27\), the desired compound. Heating \(22\) in phosphorus oxychloride produces \(16\), which is purified by crystallization and/or distillation.

During the synthesis of \(16\) a troublesome by-product was encountered, the structure of which turned out to be \(29\). During later runs we moderated the temperature of the \(26\) to \(27\) steps to avoid this problem. Condensation of \(15\) with \(16\) in the presence of triethylamine produces the chloronitro compound \(30\). Reduction of the nitro group with hydrogen and Raney nickel at atmospheric pressure produces amino compound \(31\), which must be carried on immediately to the ring-closed material. Cyclization of \(31\) is accomplished with triethyl orthoformate in \(\text{N,N-diethylacetamide}\) in the presence of HCl. In order to isolate \(32\) the crude cyclized material is treated with 50% aqueous acetic acid followed by ammonia in alcohol. These last steps remove any orthoester formed at the 2'- and 3'-hydroxyls during the triethyl orthoformate step. Conversion of \(32\) to \(6b\) is accomplished by displacement of the chlorine with hydrazine followed by N-N bond cleavage of \(33\) with Raney nickel.

With \(6b\) in hand, we began to explore the chemistry leading to the various target structures. At this point we conducted new experiments on small scale until we
15 + 16 $\xrightarrow{\text{Et}_3\text{N, EtOH}}$ [Chemical structure 30]

$\xrightarrow{\text{H}_2, \text{Raney Ni}}$ [Chemical structure 31]

1) HC(OEt$_3$)$_3$
2) DMAC, HCl
3) Aq. HOAc

$\xrightarrow{\text{NH}_2\text{NH}_2}$ [Chemical structure 33]

$\xrightarrow{\text{Raney Ni, H}_2\text{O}}$ 6b
determined the best method to obtain a new compound, and then scaled up the successful reactions. This procedure was followed for all of the chemistry that follows.

Treatment of 6b with thionyl chloride in trimethyl phosphate affords a reasonable yield of 34, which is purified by recrystallization from water. Compound 35 was both submitted for evaluation as well as used as an intermediate for making other target compounds. Dechlorination of 34 was attempted by treatment with tri-n-butyltin hydride in the presence of 2,2'-azobis (2-methylpropionitrile), a radical initiator. Heating at reflux for several days in tetrahydrofuran resulted in about a 77% conversion to 35, as judged by HPLC, with the remainder apparently being unreacted starting material. Increasing the reflux temperature by switching to dioxane as the solvent afforded little improvement in the reaction. No explanation is obvious as to why this reaction will not go to completion. After the appropriate processing, then, the 5'-deoxy compound 35 must be separated from 34. The separation is rendered difficult because compounds are inseparable on silica gel chromatography. Treatment of 34 with the sodium salt of 2-methyl-1-propanethiol in alcohol produces 36. This transformation provided the solution to the problem of the purification of 35. Treating the 34 + 35 mixture with sodium 2-methyl-1-propanethiolate converted it to a mixture of 35 and 36. The different mobility of 36 made it possible to separate this latter mixture chromatographically. This procedure allowed us to obtain some pure 35, which was submitted for screening. The synthesis of 36 from 34 appears to proceed very smoothly, and we can readily obtain material that has no obvious impurities. Examination of the $^1$H-NMR of this material allows the conclusion, however, that another material with an isobutylthio group is present. This impurity is apparently not a nucleoside, and though it is also not 2-methyl-1-propanethiol, no other protons are in evidence. Several methods were tried to remove this impurity, but these met with only partial success. As a result of these difficulties, we have not been able to submit 36 for evaluation.

In one apparently routine run converting 15 to 32, two products were formed from this sequence. The minor product was the desired 32, and the major product (by far) was identified as 37. The structure of 37 was first suggested by its mass spectrum (FAB), and then confirmed by $^1$H-NMR, $^{13}$C-NMR, and elemental analyses. Particularly noteworthy was the fact that the heterocyclic ring exhibited four signals in the $^1$H-NMR, a singlet for H-2, doublets for H-4 and H-6, and a doublet of doublets for H-7. The separation of 37 from 36 was accomplished by flash chromatography on silica gel with elution with 4:1 chloroform-methanol. The step that unexpectedly went astray must have been the Raney
nickel reduction, which apparently produced 38 instead of the compound still containing the chlorine. The hydrogenolysis of chlorines from aromatic rings is facilitated by basic conditions, and for that reason the Raney nickel is always washed to neutrality prior to use. This run was no different, so our only suggested explanation is that this particular batch of Raney nickel had a basic impurity that did not completely wash out. Of the many times the reaction has been run, this is the only time when 37 has been seen. In view of its interesting structure, we submitted 37 for screening, thus salvaging something from the time spent on this run.

During the last several months of the contract we began to examine the preparation of target compounds with the 5'-carbon oxidized. Our goal, of course, would be the synthesis of compounds such as 39e. With carbocyclic adenosine itself, access to the comparable compounds is straightforward,19 and we expected the same to be true with carbocyclic 3-deazaadenosine. Such was not the case, however, as seen in the paragraphs that follow.

In our first approach, the isopropylidene derivative 39a was prepared in 72% yield from 6b, acetone, and perchloric acid in the usual manner. A solution of 39a in dilute aqueous KOH was treated with excess KMnO4, as has been done with other nucleosides.23-25 All of the permanganate was consumed, though thin-layer chromatographic analysis showed that the main component was still starting material. Chromatographic separation and isolation of the two main organic components of the reaction mixture confirmed that the major band was still starting material. The minor band proved to be the carboxylic acid 40, which had lost the isopropylidene group. The yield of 40 was <10%, and the mass balance suggested clearly that the permanganate was degrading the starting material, so we turned to other methods.

In another approach, we wanted to repeat the KMnO4 oxidation with the 4-amino group blocked as well as the secondary OH's of 6b blocked. Compound 6b was treated with benzoyl chloride in pyridine under standard conditions. Crystallization of the crude product from EtOH gave two crops of 41 totaling 97% of the theoretical as the pentabenzoyl derivative. Brief treatment (15 min) of 41 with NaOCH3 in anhydrous MeOH/pyridine26 gave 42 in 78% yield after purification on a short silica gel column. An NMR spectrum confirmed location of the benzoyl group on the amine rather than on one of the cyclopentane hydroxyls. Treatment with a small amount perchloric acid in dry acetone by the usual procedure gave 39b in 74% yield after crystallization from water.
Several small-scale experiments with 39b were performed in an attempt to find the mildest conditions that would lead to oxidation of the primary alcohol. In one experiment KMnO₄ was added to an aqueous acetone solution of 39b with no sign of reaction. Addition of two drops of 0.1 N KOH gave no reaction. Addition of a full equivalent of KOH gave rapid consumption of permanganate as seen with 39a and precipitation of MnO₂; however, TLC showed that starting material was the only UV-active component.

In other experiments, solutions of 39b in aqueous KOH were stirred with excess KMnO₄ at room temperature and with excess KMnO₄ in an 80°C water bath. In both cases TLC showed formation of traces of the desired acid, but starting material was still the major constituent, and the strong base had caused some hydrolysis of the benzoyl blocking group, especially in the heated sample.

Because of these results we began to investigate other oxidation methods. A particularly attractive one was platinum-catalyzed oxidation by oxygen gas. In a small-scale experiment, a solution of 39b in aqueous acetone was added to a suspension of platinum black (obtained by prereduction of PtO₂) in water and buffered with NaHCO₃ to maintain the pH. This suspension was heated at 60°C while oxygen was bubbled through, resulting in the formation of the desired acid 43a. Unfortunately, this result could not be repeated on a larger scale. Variations in solvent, catalyst (both amount and batch number), temperature, and pH were all examined without success. In addition, this oxidation was attempted on 6b and 42, as well, without success. Available literature does indicate that the reaction is highly variable.²⁷

At this stage we decided to approach the target structures oxidized at C-5' by two other methods. One approach was to see if we could convert the 5'-alcohol initially to an aldehyde, which should be more readily converted to the carboxylic acid. The other approach was to build up a carbocyclic nucleoside with the carbon that would become C-5' already at the proper oxidation level.

Attempted oxidation of 39a with bis(trifluoroacetoxy)iodobenzene was unsuccessful. Treatment of 39b with pyridinium dichromate also afforded no oxidation at C-5'. The use of N,N'-dicyclohexylcarbodiimide and dimethyl sulfoxide with pyridinium trifluoroacetate as the acid catalyst, however, resulted in oxidation of 39b to the desired aldehyde 43b. The use of methanol in the chromatographic purification of 43b resulted in the actual isolation of 43b and its hemiacetal and acetal. This minor problem can be circumvented by the avoidance of alcohols in the workup and purification. The next step in this sequence, of course, would be oxidation of the aldehyde to the carboxylic acid. We
6b →

\[
\begin{align*}
\text{a) } R &= H \\
\text{b) } R &= \text{COC}_6\text{H}_5
\end{align*}
\]

\[
\begin{align*}
\text{Bz} &= \text{benzoyl(COC}_6\text{H}_5) \n\end{align*}
\]

a) \( R = \text{CO}_2\text{H} \)
b) \( R = \text{CHO} \)

16
have found that 43b can be oxidized on a small scale to 43a with m-chloroperoxybenzoic acid. If this reaction can be scaled up, it should allow us reasonable access to 43a.

In our other approach, the methyl ester 23 was condensed directly with 16 to produce the nitro compound 44. Reduction of 44 with Raney nickel followed immediately by cyclization with triethyl orthoformate and hydrochloric acid produced 45, isolated as the ethyl ester because of ester exchange. Presumably, the methyl ester could be isolated by employing trimethyl orthoformate in the cyclization step. All that remains is to replace the chlorine of 45 with an amino group, and manipulate the 5'-carbon as desired to afford structures of the 8e type.

\[
23 + 16 \rightarrow \text{CH}_3\text{O}_2\text{C} \quad \text{44} \quad \rightarrow \quad \text{CH}_3\text{CH}_2\text{O}_2\text{C} \quad \text{45}
\]

\[
\text{CH}_3\text{O}_2\text{C} \quad \text{H} \quad \text{N} \quad \text{O}_2\text{N} \quad \text{Cl} \quad \text{R}
\]

\[
\text{a) R = OCH}_3
\]

\[
\text{b) R = CH}_3\text{O}_2\text{C} \quad \text{NH}^-
\]
D. Compounds Submitted

Code No. C798-38RB  
1.5 g

Code No. C798-93RB  
0.5 g

Code No. C798-102RB  
0.5 g

E. Experimental Section

Melting points were recorded on a Mel-Temp apparatus and are uncorrected. NMR spectra were recorded on a Nicolet NMC 300NB spectrometer operating at 75.6 MHz for $^{13}$C and 300.635 for $^1$H. Chemical shifts are expressed in parts per million downfield from tetramethylsilane. Mass spectra were recorded on a Varian MAT 311A mass spectrometer in the fast atom bombardment (FAB) mode. Microanalyses were performed by the Molecular Spectroscopy Section, Southern Research Institute.

The multistep syntheses leading to compounds 6b, 15, 20, 21, 30, 16, 31 and 23, 20, 21 were carried out as described in the literature.

(±)-4-Amino-1-[(1a,2β,3β,4α)-4-(chloromethyl)-2,3-dihydroxycyclopentyl] imidazo[4,5-c] pyridine (34). (Trivial name 5'-chloro-5'-deoxycarbocyclic 3-deazaadenosine). To a cooled (ice-methanol bath) suspension of 6b (3.002 g, 9.98 mmol) in trimethyl phosphate (30 mL) was added thionyl chloride (8 mL), and the reaction was allowed to warm to room temperature and stirred overnight, during which time a yellow precipitate developed. The solution was diluted with ether, chilled, and the solid collected. This material, the 2',3'-O-cyclic sulfite of the product, was dissolved in hot water, and the solution adjusted to pH 9 with 1 N NaOH. The solution was concentrated somewhat, treated with charcoal, and filtered through Celite, washing with hot water. The volume was again reduced and the solution allowed to stand, depositing 2.107 g (74.6%) of blue-white crystals of 34, which decomposed slowly beginning at 192°C; MS (FAB) m/z 282 (M + 1); UV (H$_2$O) $\lambda_{\text{max}}$ ($\epsilon \times 10^{-3}$) pH 1, 263 nm (10.4), 270 nm (sh), pH 7, 263 nm (10.7), 270 nm (sh), pH 13, 267 nm (10.7); $^1$H NMR (Me$_2$SO-$d_6$) $\delta$ 1.70 (m, 1, H-5'), 2.26-2.46 (m, 2, H-4', H-5'), 3.72-3.92 (m,
3, H-3', 2 H-6'), 4.22 (m, 1, H-2'), 4.58 (m, 1, H-1'), 5.00 (br s, 1, 3'-OH), 5.15 (br s, 1, 2'-OH), 6.13 (br s, 2, NH₂), 6.82 (d, 1, J = 2 Hz, H-3), 7.65 (d, 1, H-2), 8.17 (s, 1, H-8); 

¹³C NMR (Me₂SO-d₆) δ 30.01 (C-5'), 45.18 (C-4'), 47.39 (C-6'), 60.12 (C-1'), 71.94, 74.56 (C-2', 3'), 97.06 (C-3), 126.81 (C-4), 137.98 (C-5), 139.91 (C-2), 139.99 (C-8), 152.30 (C-6).


(±)-4-Amino-1-[(1a,2β,3β,4α)-4-methyl-2,3-dihydroxycyclopentyl] imidazo [4,5-c] -pyridine (carbocyclic 5'-deoxy-3-deazaadenosine, 35). To a suspension of 3.04 g (10.8 mmol) of (+)-4-amino-1-[(1a,2β,3β,4α)-4-(chloromethyl)-2,3-dihydroxycyclopentyl] -imidazo [4,5-c] pyridine (carbocyclic 5'-chloro-5'-deoxyadenosine, 34) in dry THF (800 mL) was added a,a'-azobis (isobutyronitrile) (0.5 g, 3.04 mmol) and tri-n-butyltin hydride (12.52 g, 43 mmol), and the mixture heated at reflux under a nitrogen atmosphere. Additional tri-n-butyltin hydride (~12 g) was added in several portions over the course of two days. Analysis by HPLC indicated that the reaction gradually proceeded to about 80% completion, and then would proceed no further. The solvent was removed under reduced pressure, and then the residue was diluted with cold petroleum ether (35-60 °C). The solid that formed was filtered off. Attempts at obtaining pure 35 from this solid were unsuccessful. We therefore dissolved the residue in 1 N NaOCH₃ in ethanol, and added 2-methyl-1-propanethiol. The reaction mixture was heated at reflux for 5 h, after which time HPLC analysis showed the complete loss of 34 and the formation of a corresponding amount of the isobutylthio compound 36. The reaction mixture was cooled in an ice bath, adjusted to pH 6 with glacial acetic acid, and the solvent was removed under reduced pressure. The nucleosides were then extracted from the solid with hot CHCl₃. Separation of 35 and 36 was achieved by flash chromatography (100 g of silica gel for ~3 g of the mixture, elution with CHCl₃-CH₃OH, 4:1). Final purification of 35 was achieved by recrystallization from ethanol, m.p. 218-219 °C. Data for 35: ¹H-NMR (Me₂SO-d₆) δ 1.13 (d, 3, CH₃, J₄,5 = 7 Hz), 1.53, 2.33 (2 m, 2, 2 H-6'), 1.99, (m, 1, H-4'), 3.61 (m, 1, H-3'), 4.18 (m, 1, H-2'), 4.53 (m, 1, H-1'), 4.9 (br, 2, 2 OH), 6.14 (br s, 2, NH₂), 6.81 (d, 1, H-7, J₆,7 = 6 Hz), 7.66 (d, 1, H-6), 8.19 (s, 1, H-2); ¹³C-NMR (Me₂SO-d₆) δ 18.79 (CH₃), 34.01 (C-6'), 37.44 (C-4'), 61.15 (C-1'), 74.88, 76.07 (C-2',3'), 97.00 (C-7), 126.79 (C-7a), 138.05 (C-3a), 139.88, 139.92 (C-2,6), 152.27 (C-4).

Anal. Calcd for C₁₂H₁₅ClN₄O₂·0.25 EtOH: C, 57.79; H, 6.79; N, 21.57. Found: C, 57.45; H, 6.85; N, 21.66. Ethanol in the proper amount was seen in the ¹H-NMR of the sample.
Formation and Isolation of (±)-1-[(1α,2β,3β,4α)-4-(hydroxymethyl)-2,3-dihydroxy-
cyclopentyl] imidazo[4,5-c] pyridine (carbocyclic 3-deazapurine ribonucleoside, 37) as an
Alternate Product From One Run of the Synthesis of Carbocyclic 3-Deazaadenosine. The
synthesis of carbocyclic 3-deazaadenosine (6b), as noted in the body of the report,
proceeds through 30 and 32 using procedures that have already been reported. The
reaction mixture at the end of the two-step sequence to produce 32 instead contained
mainly 37. Separation of 37 from 32 was accomplished by flash chromatography (7 g of
crude residue, 150 g of silica gel, elution with CHCl₃-CH₃OH, 4:1). The appropriate
column fractions, on standing in methanol deposited 37, m.p. 196-197 °C.

Data on 37: UV (H₂O) λ max (ε) pH 1, 264 (4.35), 255 (sh); pH 7, 270 (sh), 262.5
(4.44), 255 (4.76); pH 13, 270 (sh), 262.5 (4.55), 255 (5.04); ¹H-NMR (Me₂SO-d₆) δ 1.78,
2.34 (2 m, 2, 2 H-6), 2.11 (m, 1, H-4), 3.53 (m, 2, 5'-CH₂), 3.85 (m, 1, H-3),
4.14 (m, 1, H-2'), 4.75 (m, 1, H-1'), 4.86, 5.05, and ca. 4.76 (hidden) (3 br s, 3, OH's), 7.74 (dd, 1, H-7,
J₆,₇ = 5 Hz, J₄,₇ = 1 Hz), 8.34 (d, 1, H-6), 8.51 (s, 1, H-2), 8.97 (d, 1, H-4); ¹³C-NMR
(Me₂SO-d₆) δ 28.32 (C-6'), 45.10 (C-4'), 60.43 (C-1'), 62.65 (C-5'), 71.90, 75.12 (C-2',3'),
106.79 (C-7), 138.24 (C-3a), 140.87 (C-7a), 140.98 (C-6), 141.89 (C-4), 144.25 (C-2).

Anal. Calcd for C₁₂H₁₅N₃O₃: C, 57.82; H, 6.07; N, 16.86. Found: C, 57.59; H,
6.47; N, 16.51.

(±)-4-Amino-1-[(1α,2β,3β,4α)-4-(hydroxymethyl)-2,3-(isopropylidenedioxy)cyclo-
pentyl] imidazo[4,5-c] pyridine (39a). A solution of dry acetone (40 mL), 70% perchloric
acid (0.2 mL), and 2,2-dimethoxypropane (2 mL) was stirred at room temperature for 5
min. Solid 6b·hydrochloride (301 mg, 1.0 mmol) was added, and the resulting solution was
stirred for 2 h. With cooling in an ice bath, dry pyridine (2 mL) was added, and the
mixture was evaporated to dryness in vacuo. A solution of the residue in H₂O (40 mL) was
adjusted to pH 10 with 1 N NaOH to liberate the amine free base, and then it was
extracted with a small volume of CH₂Cl₂. Evaporation of the dried (MgSO₄) extract gave
only a small solid residue (56 mg, 18.4%), but during the extraction, white crystalline solid
began to deposit on the glass walls in the water layer. After cooling in the refrigerator,
this solid was collected by filtration, washed with cold water, and dried in vacuo: yield
162 mg (53.3%).

Based on thin-layer chromatography and mass spectral evidence (FABMS m/z 305
(M + 1)⁺) both crops were judged to be suitable for use as an intermediate without further
treatment.
(+)-4-N,N-Dibenzoylamino-1-\{(1a,2\beta,3\beta,4a)-2,3-dibenzoyloxy-4-(benzoyloxymethyl)-cyclopentyl\} imidazo[4,5-c] pyridine (41). Benzoyl chloride (0.70 g, 5.0 mmol) was added dropwise to a stirred ice-cold suspension of 6b-hydrochloride (301 mg, 1.0 mmol) in dry pyridine (20 mL). After stirring for 18 h at room temperature, additional benzoyl chloride (1.0 mmol) was added, and the mixture was heated on a 50°C water bath for 2 h to complete the reaction. Pyridine was evaporated in vacuo. A solution of the residue in CHCl₃ (50 mL) was washed by shaking with dilute HCl, saturated NaHCO₃ solution, and water, dried over Na₂SO₄ and evaporated to dryness. Toluene (50 mL) was added and evaporated to aid in removal of a trace of pyridine. A solution of the residue in hot EtOH (175 mL) deposited white crystals that were collected in two crops totaling 76 mg (97% as the pentabenzoyl derivative). The larger crop (722 mg, mp 150-151°C with prior sintering) was homogeneous by TLC in two solvent systems, but mass spectral analysis (FAB) showed a peak at m/z 681 (M + 1)⁺ corresponding to the tetrabenzoyl derivative along with the very strong peak at 785 (M + 1)⁺ corresponding to the expected pentabenzoyl compound.

(+)-4-N-Benzoylamino-1-\{(1a,2\beta,3\beta,4a)-2,3-dihydroxy-4-(hydroxymethyl)cyclopentyl\} -imidazo[4,5-c] pyridine (42). A small quantity (~50 mL bed volume) of Dowex 50W-X8 cation exchange resin (H⁺ form) was converted to the pyridinium form in a glass column by washing first with 200 mL of 10% pyridine/H₂O and then with several column volumes of water.

A solution of sodium methoxide in dry MeOH (4.6 mL of 1N, 4.6 mmol) was added at room temperature to a stirred solution of 41 (714 mg, 0.91 mmol) in dry pyridine (17 mL) and methanol (5 mL). After 10 min at room temperature, the reaction mixture was cooled in ice water, stirred for 5 min more, and then treated all at once with a suspension of ~10 mL (bed volume) of the pyridinium resin in 5 mL of water. There was an induction period of ~5 min before any change in pH was observed, but suddenly the mixture fell from pH 11.5 (meter) to pH 7.5. Addition of 5 mL more resin in 5 mL of H₂O and stirring for 10 min only decreased the mixture to pH 7.3. The resin was filtered off, washed with hot MeOH/H₂O (3:1, 50 mL), and the combined filtrate and wash were evaporated in vacuo to a white foam. A solution of this in CHCl₃/MeOH (4:1) was applied to a very short column (~5 cm high) of silica gel (E. Merck, 230-400 mesh) in order to remove benzoic acid and pyridine hydrochloride contaminants. The product-containing fractions were pooled and evaporated. Attempts to crystallize the foam from MeOH/H₂O and from isopropyl alcohol were both successful, but solubility losses were too high. Finally, all solvents were evaporated; the solid was dissolved in MeOH (20 mL), and Et₂O (200 mL) was added
very slowly dropwise until crystallization was induced, and it was allowed to proceed spontaneously. When it appeared complete, the rest of the Et₂O was added slowly. The solid was collected, washed with Et₂O, and dried in vacuo over P₂O₅: yield 260 mg (77.6%); mp 163-164°C with prior sintering. Mass spectral analysis (FAB) confirmed that this was the desired monobenzoyl compound (m/z 369, M + 1)⁺, and a pmr spectrum confirmed that the location of the benzoyl was on the amino group.

(±)-4-N-Benzoylamino-1-[(1a,2β,3β,4a)-4-hydroxymethyl-2,3-(isopropylidenedioxy)cyclopentyl]imidazo[4,5-c]pyridine (39b). A mixture of dry acetone (60 mL), 70% perchloric acid (0.2 mL), and 2,2-dimethoxypropane (2.5 mL) was stirred at room temperature for a few min. Solid 42 (240 mg, 0.65 mmol) was added, and the solution was stirred for one hour, then cooled in an ice bath and treated with 6 mL of dry pyridine. Evaporation to dryness in vacuo gave a white solid residue which was extracted by vigorous magnetic stirring with CHCl₃ (100 mL). Filtration gave a clear, colorless filtrate that contained very little product (TLC). The CHCl₃-insoluble solid was suspended in H₂O (20 mL), and solid NaHCO₃ was added in small portions until the mixture was adjusted to pH 7.3. Most of the insoluble material dissolved during the early stage of the addition, but as neutrality was approached, white solid began to precipitate. After cooling in ice, the solid was collected by filtration, washed with cold water, and dried in vacuo over P₂O₅: yield 197 mg (74.2%); mp 201-202°C; FABMS m/z 409 (M + 1)⁺.

(±)-4-(1a,2β,3β,4a)-(4-Benzoylamino)imidazo[4,5-c]pyridin-1-yl-2,3-(isopropylidenedioxy)cyclopentanecarboxylic Acid (43a). A suspension of PtO₂ (50 mg, Engelhard) in H₂O (25 mL) was stirred in a hydrogen atmosphere for one hour. The resulting suspension of platinum was warmed in a 60°C water bath while oxygen was bubbled vigorously through the magnetically stirred mixture. After bubbling for 10 min, a solution of the alcohol 39b (41 mg, 0.1 mmol) in H₂O (5 mL) and acetone (3 mL) was added, followed by solid NaHCO₃ (50 mg). Bubbling was continued for one hour, after which TLC showed an insignificant amount of remaining starting material. The catalyst was filtered off and washed with hot water. The filtrate was adjusted to pH 5 with 1 N HCl, and the mixture of the carboxylic acid 43b and NaCl was evaporated to dryness: wt. 71 mg; FABMS m/z 423 (M + 1)⁺, 445 (M + Na)⁺.

This pilot sample was kept without further treatment for use as a chromatographic reference in later attempts to repeat this reaction on a preparative scale.
(±)-4-(1a,2β,3β,4α)-[4-(Benzoylamino)imidazo[4,3-b]pyridin-1-yl]-2,3-(isopropylidenedioxy)cyclopentanecarboxaldehyde, mixture with methylhemiacetal and dimethylacetal (43b). Under an atmosphere of dry \( \text{N}_2 \), a solution of \( \text{N},\text{N}'\)-dicyclohexylcarbodiimide (382 mg, 1.85 mmol) in dry dimethylsulfoxide (4.6 mL) was added to a solution of 39b (252 mg, 0.62 mmol) in DMSO (10 mL) at room temperature. To this was added a solution of pyridinium trifluoroacetate (60 mg, 0.31 mmol) in DMSO (1.5 mL), and after stirring for ~2 min, a precipitate of \( \text{N},\text{N}'\)-dicyclohexylurea (DCU) began to deposit. After 24 h, TLC showed only a trace of starting material, so with cooling in an ice bath the reaction mixture was diluted with \( \text{H}_2\text{O} \) (10 mL) and stirred for 15 min to hydrolyze excess DCC. The deposit of DCU was filtered off, washed with cold water, and dried in vacuo: weight was 371 mg, 89% of theoretical. The filtrate was evaporated in vacuo (oil pump, glass lyophilizer cooled in dry ice) to a glass: yield 244 mg.

In order to separate the product from a little DCU and a small quantity of starting material, a solution of the glass in \( \text{CHCl}_3/\text{MeOH} \) (93:7) was chromatographed on a short silica gel column (E. Merck, 230-400 mesh). The product-containing fractions gave a white brittle foam (222 mg) on evaporation of solvents that showed three spots by TLC, and all three gave a positive aldehyde test with a spray containing 2,4-dinitrophenylhydrazine and conc. HCl in EtOH. Mass spectral analysis (FAB) identified the three components as the desired aldehyde, m/z 407 (M + 1)\(^+\), and the corresponding methylhemiacetal, 439 (M + 1)\(^+\), and dimethylacetal, 453 (M + 1)\(^+\), derivatives. Presumably, the last gives a positive spray test because of hydrolysis on the plate by HCl in the spray.

(±)-Methyl (1a,2β,3β,4α)-4-[(2-chloro-3-nitro-4-pyridyl)amino]-2,3-dihydroxy-cyclopentanecarboxylate (44). To a solution of 23 (4.0 g, 19 mmol) and 16 (11.0 g, 57 mmol) in dry methanol (900 mL) was added triethylamine (9.6 mL, 67.2 mmol), and the solution was heated at reflux for 3 d. After evaporation of solvent, excess 16 was removed by trituration with petroleum ether. The remaining residue, after drying, was purified by flash chromatography (silica gel, 2" ID column, 310 g) eluting with 97:3 \( \text{CHCl}_3-\text{CH}_3\text{OH} \). Appropriate fractions were pooled, evaporated to dryness, leaving 3.81 g (60.5%) of 44, which was used directly in the next steps. FABMS m/z 332 (M + 1)\(^+\). The major impurity isolated from this column (~15-20%) was identified tentatively [FABMS m/z 471 (M + 1)\(^+\)] as 46b, and 46a was tentatively identified as one of the other minor (~1%) impurities [FABMS m/z 328 (M + 1)\(^+\)]. \(^1\)H-NMR (44, Me\(_2\)SO-\( \text{d}_6 \)) δ 1.09, 2.34 (2 m, 2, 2 H-6'), 2.75 (m, 1, H-4'), 3.63 (s, 3, CH\(_3\)), 3.72 (m, 1, H-2'), 3.82 (m, 1, H-1'), 4.00 (m, 1,
H-3'), 5.01, 5.02 (2 d, 2, 2',3'-OH, J = 6 Hz), 7.01 (d, 1, H-5), J = 6 Hz), 7.25 (d, 1, NH, J = 7 Hz), 8.02 (d, 1, H-6).

(±)-Ethyl (1α,2β,3β,4α)-4-{4-chloroimidazo [4,3-b] pyridin-1-yl}-2,3-dihydroxy-cyclopentanecarboxylate (45). To a solution of 44 (83 mg, 0.25 mmol) in methanol (50 mL) was added ca. 20 mg of Raney nickel. The mixture was stirred until hydrogen uptake was complete (5-6 h), and the catalyst was filtered off (Celite), washing with hot ethanol. The filtrate was evaporated to dryness under reduced pressure and then immediately dissolved in N,N-diethylacetamide (4 mL). To this solution were added triethyl orthoformate (8 mL) and conc. HCl (0.2 mL), and the reaction allowed to stir at room temperature overnight. Solvent was removed under reduced pressure, and the residue was dissolved in 50% aqueous acetic acid and stirred at room temperature for 4 h. After removal of the solvent under reduced pressure, water was added to the residue and the solution was evaporated to dryness again to ensure removal of the acetic acid. The residue was taken up in 10% ammoniacal methanol and stirred at room temperature for 4 h. After removal of the solvent, 45 was purified by passage through a small silica gel column, eluting with 9:1 CHCl₃-CH₃OH, affording 55 mg (70%) of 45, FABMS m/z 326 (M + 1)+.

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