Structure−Activity Relationship of Heterobase-Modified 2′-*C*-Methyl Ribonucleosides as Inhibitors of Hepatitis C Virus RNA Replication

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Hepatitis C virus infection constitutes a significant health problem in need of more effective therapies. We have recently identified 2′-*C*-methyladenosine and 2′-*C*-methylguanosine as potent nucleoside inhibitors of HCV RNA replication in vitro. However, both of these compounds suffered from significant limitations. 2′-*C*-Methyladenosine was found to be susceptible to enzymatic conversions by adenosine deaminase and purine nucleoside phosphorylase, and it displayed limited oral bioavailability in the rat. 2′-*C*-Methylguanosine, on the other hand, was neither efficiently taken up in cells nor phosphorylated well. As part of an attempt to address these limitations, we now report upon the synthesis and evaluation of a series of heterobase-modified 2′-*C*-methyl ribonucleosides. The structure−activity relationship within this series of nucleosides reveals 4-amino-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine and 4-amino-5-fluoro-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine as potent and noncytotoxic inhibitors of HCV RNA replication. Both 4-amino-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine and 4-amino-5-fluoro-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine display improved enzymatic stability profiles as compared to that of 2′-*C*-methyladenosine. Consistent with these observations, the most potent compound, 4-amino-5-fluoro-7*H*-pyrrolo[2,3-*d*]pyrimidine ribonucleoside, is orally bioavailable in the rat. Together, the potency of the 2′-*C*-methyl-4-amino-pyrrolo[2,3-*d*]pyrimidine ribonucleosides and their improved pharmacokinetic properties relative to that of 2′-*C*-methyladenosine suggests that this class of compounds may have clinical utility.

Introduction

Hepatitis C virus (HCV) infection constitutes a serious health problem in need of more efficacious therapies. Current estimates suggest that about 170 million people suffer from HCV infection worldwide. Although HCV infection is often asymptomatic, it can progress to chronic hepatitis, leading to liver cirrhosis and hepatocellular carcinoma in a significant percentage of patients. Combination therapy using pegylated interferon-α and ribavirin have significantly improved the sustained response rates for patients infected with HCV genotypes 2 or 3, but the response rate for patients infected with genotype 1 remains at a disappointing 42−46%.[1]

HCV encodes a series of viral proteins including the NS2/3 autoprotease, the NS3 serine protease/NTPase/helicase, and the NS5B RNA-dependent RNA polymerase (RdRp).[2−4] The RdRp, encoded within the 3′-terminal portion of the HCV genome, is essential for viral replication and therefore represents a valid target for therapeutic intervention through the design of specific inhibitors. Recently, a number of nucleoside inhibitors (NIs)[5−9] and non-nucleoside inhibitors (NNIs)[10−13] of the NS5B polymerase have been described. Although NIs are dependent on the metabolism of the nucleoside to the corresponding triphosphates for their activity, they possess advantages over the NNIs in that the mode of action (chain termination) is well defined and can be easily verified. Moreover, NIs acting by chain termination must, by mechanistic necessity, bind in the highly conserved active-site region of HCV NS5B and thus have a high likelihood of affecting RNA replication for all HCV genotypes.

We recently reported the synthesis and structure−activity relationship (SAR) of purine ribonucleosides as inhibitors of HCV RNA replication.[8] In this study, 2′-*C*-methyladenosine and 2′-*C*-methylguanosine (Figure 1) were identified as potent, specific inhibitors of HCV RNA replication in cell culture, and their corresponding triphosphates were shown to be inhibitors of HCV NS5B-mediated RNA synthesis. In line with these findings, 2′-*C*-methyladenosine and 2′-*C*-methylcytidine were described as specific attenuators of HCV RNA levels by Stuyver et al.[9] We found the SAR of the 2′- and 3′-ribonucleoside positions to be quite stringent; however, HCV NS5B appeared to be somewhat amenable to the modification of the purine heterobase.[8]

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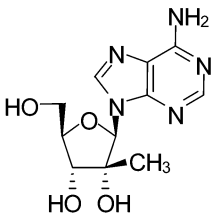
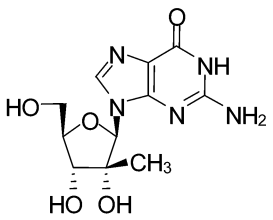
	 1	 2
IC_{50},HCV NS5B/μM	1.9	0.13
EC_{50},HCV Replicon/μM	0.26	3.5
Intracellular NTP/pmol per 10^6 cells	105	0.2
ADA Substrate	yes	-
PNP Substrate	yes	no
Oral bioavailability in rat (%F)	0	85

Figure 1. Structure of nucleosides **1** and **2**, inhibitory potency of the corresponding nucleoside 5′-triphosphates on HCV NS5B-mediated RNA synthesis (HCV NS5B, $IC_{50}/\mu M$), inhibitory potency of nucleosides on HCV RNA replication in a subgenomic replicon assay harbored in HB-1 cells (HCV replicon, $EC_{50}/\mu M$), cellular uptake and metabolism to triphosphate in Huh-7 cells, and oral bioavailability (%*F*).

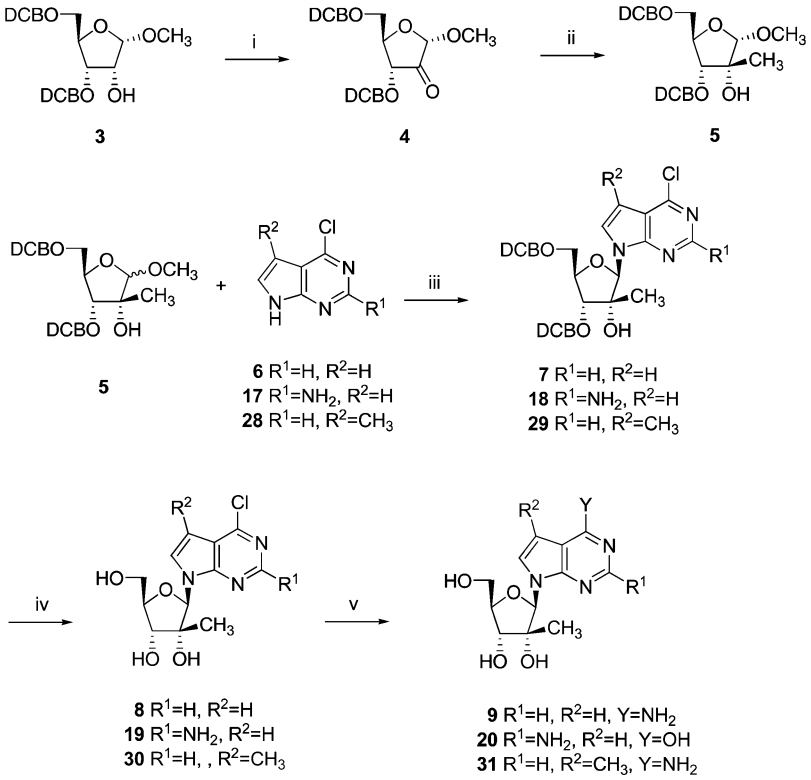
Several NTPs containing heterobase modifications were found to be inhibitors of HCV-mediated RNA synthesis, whereas the corresponding nucleosides generally failed to inhibit HCV RNA replication in the cell-based, subgenomic replicon assay.[8] These data suggest that cellular uptake and/or kinase mechanisms are highly sensitive to modifications in the heterobase of ribonucleosides. A closer look at the two most potent NIs indicated that, for 2′-*C*-methylguanosine, cellular uptake and intracellular metabolism to the corresponding triphosphate were inefficient, suggesting that the potency of this NI may be limited in vivo.[7] Furthermore, 2′-*C*-methyladenosine was found to be a substrate for adenosine deaminase (ADA)-mediated and purine nucleoside phosphorylase (PNP)-mediated conversions, suggesting that the high clearance rate and lack of oral bioavailability observed for this NI[7] may be due in part to metabolic conversion.[8]

As part of an effort to address the limitations of 2′-*C*-methyladenosine (**1**) (Figure 1) with respect to its enzymatic stability and bioavailability, we report here the synthesis of a series of heterobase-modified 2′-*C*-methylribonucleosides and evaluate their potency as inhibitors of HCV RNA replication. Our analysis of these heterobase-modified 2′-*C*-methylnucleosides leads to the identification of 4-amino-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine and 4-amino-2-fluoro-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine as potent, noncytotoxic inhibitors of HCV RNA replication. Furthermore, these nucleosides are found to possess attractive profiles in terms of enzymatic stability when compared to that of 2′-*C*-methyladenosine. Most importantly, as exemplified by the more potent fluoro-substituted derivative, this class of inhibitors possesses a pharmacokinetic profile that may allow oral dosing of this compound.

Chemistry

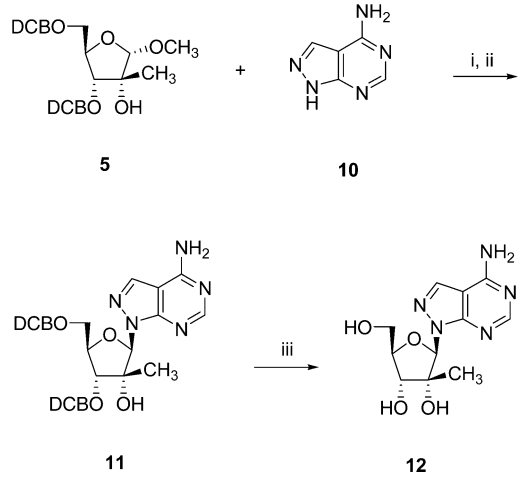
The effect of the modification of the heterobase of 2′-*C*-methyladenosine was investigated by first synthesizing the corresponding 7-deaza derivative, 4-amino-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**9**), according to the route depicted in Scheme 1. Starting with 3,5-bis-*O*-(2,4-dichlorophenylmethyl)-1-*O*-methyl-α-D-ribofuranose (**3**), oxidation of the 2-hydroxyl group was performed using Dess−Martin periodinane in dichloromethane to give **4**. Subsequent stereospecific addition of a methyl group on the *β* face of the ribofuranose to give 3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-1-*O*-methyl-α-D-ribofuranose (**5**) was accomplished by reaction with methylmagnesium bromide in diethyl ether. The ribofuranose **5** was next converted to the corresponding 1-bromo compound (with hydrogen bromide/acetic acid in dichloromethane) and then reacted with the sodium salt of 4-chloro-1*H*-pyrrolo[2,3-*d*]pyrimidine (**6**)[14] (formed with sodium hydride) in acetonitrile to yield exclusively the *β* anomer, **7**. The removal of the dichlorophenylmethyl protection groups was performed using boron trichloride in dichloromethane to give 4-chloro ribonucleoside **8**. This compound was converted to the desired 4-amino derivative, **9**, by ammonolysis at elevated temperature. The 8-aza-7-deaza derivative of 2′-*C*-methyladenosine, 4-amino-1-(2-*C*-methyl-*β*-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (**12**), was also synthesized from ribofuranose **5**, again through the formation of the 1-bromo derivative and reaction with the sodium salt of 4-amino-1*H*-pyrazolo[3,4-*d*]pyrimidine (**10**) (formed by sodium hydride) in 1-methyl-2-pyrrolidinone to give **11** (Scheme 2). The deprotection of **11** was carried out with boron trichloride in dichloromethane to give the desired ribonucleoside, **12**. The synthesis of the 3,7-dideaza derivative of 2′-*C*-methyladenosine, 4-amino-1-(2-*C*-methyl-*β*-

Scheme 1[a]



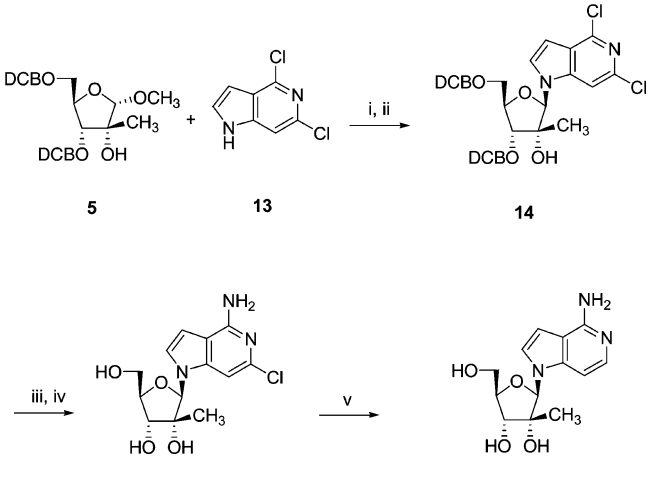
[a] Reagents and conditions: (i) Dess−Martin periodinane in dichloromethane 3 days at 0 °C to room temperature; (ii) MeMgBr in diethyl ether 7 h at −55 to −15 °C; (iii) HBr/acetic acid in dichloromethane, 4 h at 0 °C to room temperature; then sodium salt of **6** in acetonitrile, 24 h at room temperature (for **7**) or sodium salt of **17**, KOH, tris[2-(2-methoxyethoxy)ethyl]amine in acetonitrile, 1 h at room temperature (for **18**) or sodium salt of **28** in acetonitrile, 24 h at room temperature (for **29**); (iv) boron trichloride in dichloromethane 5.5 h at −78 to −20 °C; (v) methanolic ammonia, 14 h at 85 °C (for **9**) or 1 N NaOH, 5 h at reflux (for **20**) or methanolic ammonia, 14 h at 100 °C (for **31**).

Scheme 2[a]



[a] Reagents and conditions: (i) hydrogen bromide in dichloromethane, 10 min at room temperature; (ii) 4-amino-*1H*-pyrazolo[*3,4-d*]pyrimidine and NaH in 1-methyl-2-pyrrolidinone, 30 min at room temperature; (iii) boron trichloride in dichloromethane 2 h at −78 °C to −30 °C.

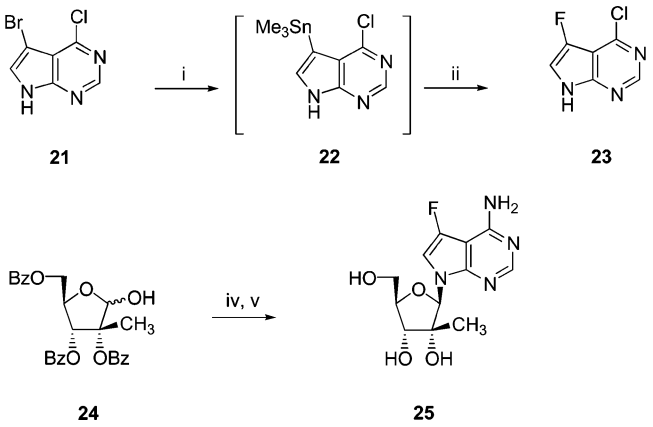
Scheme 3[a]



[a] Reagents and conditions: (i) hydrogen bromide in dichloromethane, 30 min at 0 °C; (ii) 4,6-dichloro-*1H*-pyrrolo[2,3-*c*]pyridine and NaH in 1-methyl-2-pyrrolidinone, 60 min at room temperature; (iii) boron trichloride in dichloromethane 2 h at −78 to −30 °C; (iv) liquid ammonia and CuI, 24 h at 130 °C; (v) Pd/C (10%) and concentrated aqueous ammonia in methanol under hydrogen (1 atm).

D-ribofuranosyl)-1*H*-pyrrolo[3,2-*c*]pyridine (**16**), was carried out using a similar strategy (Scheme 3). Briefly, the bromo derivative of **5** was coupled with the anion of dichloro-1*H*-pyrrolo[3,2-*c*]pyridine (**13**)[15] to yield ribonucleoside **14**. Removal of the dichlorophenylmethyl protection groups and ammonolysis were performed with boron trichloride in dichloromethane and liquid ammonia/CuI,[16] respectively, to give 4-amino-6-chloro derivative **15**. Catalytic hydrogenation with Pd/C in the presence of aqueous ammonia gave the desired compound, **16**. The analogous guanosine derivative of **9**, 2-amino-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo-[2,3-*d*]pyrimidin-4(3*H*)-one (**20**), was synthesized according to the procedure in Scheme 1 using 2-amino-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (**17**) as the heterobase.

Scheme 4[a]

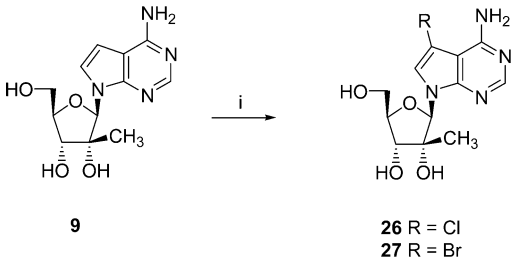


[a] Reagents and conditions: (i) *n*-BuLi in tetrahydrofuran, 30 min at −78 °C; (ii) Me_3SnCl in tetrahydrofuran, 24 h at room temperature; (iii) SELECTFLUOR in acetonitrile, 7 h at room temperature; (iv) **23**, triphenylphosphine, and DEAD in tetrahydrofuran, 24 h at room temperature; (v); liquid ammonia and dioxane, 24 h at 85 °C.

Treatment of protected ribonucleoside **18** with boron trichloride gave **19**, which was converted to the desired compound, **20**, through basic hydrolysis of the 4-chloro functionality.

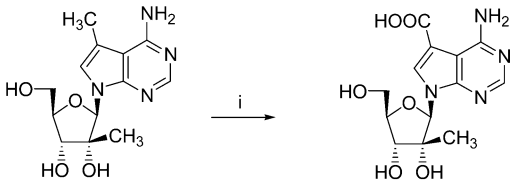
In order to examine the effect of substitution at the 5 position of the pyrrolo[2,3-*d*]pyrimidine heterobase, a series of derivatives (**25**−**27** and **31**−**34**) were prepared. The synthesis of the 5-fluoro derivative (**25**) was accomplished through the synthesis of the fluoro-substituted heterobase, 4-chloro-5-fluoro-1*H*-pyrrolo[2,3-*d*]pyrimidine (**23**, Scheme 4). Compound **23** could subsequently be coupled to the 2-*C*-methylribose. Lithiation of 5-bromo-4-chloro-1*H*-pyrrolo[2,3-*d*]pyrimidine[17] and subsequent quenching of the resulting dianion with trimethylstannane chloride gave the desired 5-trimethylstannane, **22**. This was then reacted in situ with 1-(chloromethyl)-4-fluoro-1,4-diazoniabicyclo[2.2.2]-octane bis(tetrafluoroborate) (SELECTFLUOR) to give the desired 5-fluoro derivative, **23**. A variety of conditions for glycosylation of this heterobase were examined, including coupling via the herein described bromo derivative of **5**. None of these conditions gave the desired compound in appreciable yield. However, the coupling of **23** with previously described 2,3,5-tri-*O*-benzoyl-2-*C*-methyl-D-ribofuranose[18] proceeded under Mitsunobu conditions (triphenylphosphine and diethyl azodicarboxylate in tetrahydrofuran) to give the desired *N*-7 glycosylated compound as a mixture of α and *β* anomers as well as the *N*-1 isomeric derivatives (Scheme 4). This mixture was deprotected directly using liquid ammonia at elevated temperature and subsequently separated by preparative HPLC (see general methods) to give the desired *N*-7 glycosylated *β* isomer **25**. Electrophilic substitutions on 4-amino-7-(*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine are known to yield the corresponding 5-substituted derivatives (corresponding to the purine *N*-7 position).[19] Hence, for the synthesis of 4-amino-5-chloro-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**26**), compound **9** was reacted with 1 equiv of *N*-chlorosuccinimide in dimethylformamide (Scheme 5). The bromo derivative **27** was prepared from **9** by a reaction with *N*-bromosuccinimide under similar conditions. 4-Amino-5-methyl-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**31**) was synthesized by a similar procedure to that used for **9** and **20** (Scheme 1). Briefly, 4-chloro-5-methyl-1*H*-pyrrolo[2,3-*d*]pyrimidine (**28**) was prepared according to known procedures.[19] The sodium salt of this compound (formed with sodium hydride) was reacted with the 1-bromo compound, prepared from ribofuranose **5**, in acetonitrile to give exclusively the *β* anomer. The intermediate **29** was subsequently deprotected with boron trichloride in dichloromethane to give chloro derivative **30**. Finally, ammonolysis was carried out using methanolic ammonia at elevated temperature to furnish the desired 2′-*C*-methylribonucleoside **31**. Two additional derivatives, 4-amino-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (**32**) and 4-amino-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine-5-carboxamide (**33**), were synthesized as previously described.[20] The 5-carboxylic acid derivative **34** was prepared from methyl derivative **31** by oxidation with a mixture of aqueous and methanolic ammonia and hydrogen peroxide as the oxidant (Scheme 6).

Scheme 5[a]



[a] Reagents and conditions: (i) *N*-chlorosuccinimide in DMF, 1 h at room temperature (for **25**) or *N*-bromosuccinimide in DMF, 20 min at 0 °C, and then at room temperature for 10 min (for **26**).

Scheme 6[a]

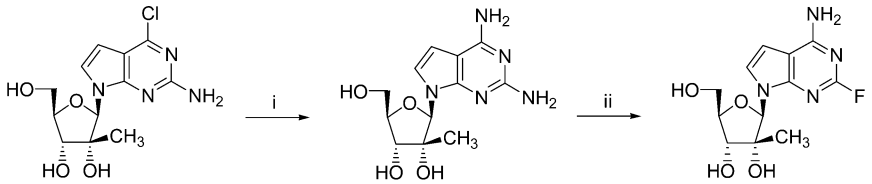


[a] Reagents and conditions: (i) aqueous ammonia, methanolic ammonia and hydrogen peroxide (30%), at room temperature for 18 h.

To evaluate the effect of substitution of the 2-position of the active 4-amino-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**9**), the corresponding 2-amino and 2-fluoro derivatives were synthesized according to the route shown in Scheme 7. Thus, 2,4-diamino-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-1*H*-pyrrolo[2,3-*d*]pyrimidine (**35**) was readily obtained from **19** by ammonolysis at elevated temperature. Subsequent, the selective transformation of the more activated 2-amino group at low temperature yielded 4-amino-2-fluoro-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-1*H*-pyrrolo[2,3-*d*]pyrimidine (**36**).

To evaluate the effect of changes in the 5 position of pyrrolo[2,3-*d*]pyrimidine derivative **20**, we synthesized its 5-chloro derivative through a two-step transformation of **19**, first with *N*-chlorosuccinimide in dimethylformamide to give the 5-chloro derivative and then by

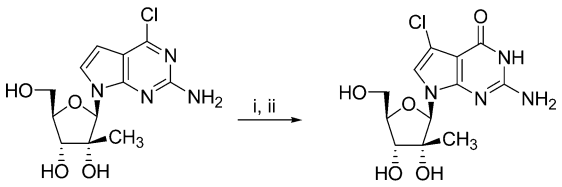
Scheme 7[a]



19 **35** **36**

[a] Reagents and conditions: (i) concentrated aqueous ammonia, overnight at 100 °C; (ii) HF in pyridine and *tert*-butyl nitrite at −25 °C.

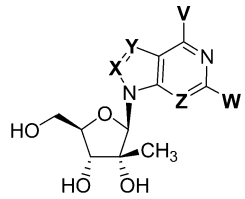
Scheme 8[a]



19 **37**

[a] Reagents and conditions: (i) *N*-chlorosuccinimide in DMF, 2 h at room temperature; (ii) 2 N aqueous NaOH, 2 h at reflux.

Table 1. Inhibitory Potency (EC_{50}) of Heterobase Modified Nucleosides on HCV RNA Replication in a Subgenomic Replicon Harbored in HB-1 Cells and Cytotoxicity (CC_{50}) as Measured by MTS



compd	X	Y	Z	V	W	HCV replicon [EC_{50}] (μM)	MTS [CC_{50}] (μM)
1	CH	N	N	NH_2	H	0.26	>100
2	CH	N	N	OH	NH_2	3.5	>100
9	CH	CH	N	NH_2	H	0.25	>100
12	N	CH	N	NH_2	H	>100	>100
16	CH	CH	CH	NH_2	H	>100	>100
20	CH	CH	N	OH	NH_2	>100	>100
25	CH	CF	N	NH_2	H	0.07	>100
26	CH	CCl	N	NH_2	H	0.13	~50
27	CH	CBr	N	NH_2	H	0.24	40
31	CH	CMe	N	NH_2	H	34	>100
32	CH	CCN	N	NH_2	H	3.1	~50
33	CH	$CCONH_2$	N	NH_2	H	0.08	~33
34	CH	CCOOH	N	NH_2	H	70	>100
35	CH	CH	N	NH_2	NH_2	100	>100
36	CH	CH	N	NH_2	F	100	>100
37	CH	CCl	N	OH	NH_2	>100	>100

alkaline hydrolysis to give 2-amino-5-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (**37**) (Scheme 8).

Results

A series of nucleosides modified in the purine heterobase were synthesized and screened in a cell-based, subgenomic replicon assay[5] for their ability to inhibit HCV RNA replication (Table 1). In addition, cytotoxicity was evaluated in parallel in an MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium]-based assay.[5] In an initial set of compounds, the heterobase of the previously identified inhibitor, 2′-*C*-methyladenosine (**1**), was modified by the transfer or replacement of one or more of the non-hydrogen-bond-forming endocyclic nitrogens with carbon to give 4-amino-pyrrolo[2,3-*d*]pyrimidine (**9**), 4-amino-pyrazolo[3,4-*d*]pyrimidine (**12**), and 4-amino-pyrrolo[3,2-*c*]pyridine (**16**) substituted 2′-*C*-methylribonucleosides. One of these nucleosides, **9**, in which the *N*-7 nitrogen of the purine had been replaced with carbon, displayed potent inhibition of HCV RNA replication in the cell-based assay (EC_{50} = 0.25 μM). The other two nucleosides, **12**, in which *N*-7 was transferred to the 8 position, and **16**, in which both the purine *N*-7 and *N*-3 had been replaced with carbon, had no effect on intracellular HCV RNA levels (EC_{50} > 100 μM). Because 2′-*C*-methylguanosine (**2**) was also identified as an active inhibitor of HCV RNA replication in our previous investigation of purine nucleosides, we decided to evaluate the corresponding guanosine mimic of **9**, 2-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (**20**). This compound tested inactive in the cell-based assay (EC_{50} > 100 μM). To gain a better understanding of the reason for the discrepancy between the inhibitory activities of **9** and **20**, we synthesized and evaluated the corresponding triphosphates as inhibitors of HCV NS5B-mediated RNA synthesis.[5] The triphosphates of both **9** and **20** were found to be potent inhibitors of RNA synthesis mediated by HCV NS5B (both yielding IC_{50} values of 0.12 μM).

On the basis of the finding that the potency of **9** is largely unchanged relative to its 7-aza derivative **1** (EC_{50} = 0.26 and 0.25 μM, respectively), we elected to further explore the structural space around the purine 7 position (corresponding to the pyrrolo[2,3-*d*]pyrimidine 5-position). The effect of relatively small, electronegative substituents such as fluoro, chloro, and bromo was investigated by evaluation of the 5-fluoro (**25**), 5-chloro (**26**), and 5-bromo (**27**) derivatives. Potent inhibition of HCV RNA replication (EC_{50} = 0.07, 0.13, and 0.24 μM, respectively) was observed upon treatment with these compounds. The potency of the 5-halogen-substituted NIs declined with increasing atomic radius and decreasing electronegativity of the 5-substituent, whereas a concomitant increase in cytotoxicity was observed (CC_{50} (24 h) > 100, ~50, and 40 μM for **25**, **26**, and **27**, respectively) with significant increases in toxicity for the 5-chloro and 5-bromo derivatives during extended culture times. For the most potent derivative (**25**), we evaluated the ability of the corresponding triphosphate to inhibit RNA synthesis mediated by HCV NS5B. The triphosphate of **25** was found to be about equipotent with the triphosphate of unsubstituted **9** (IC_{50} = 0.19 and 0.12 μM, respectively).

To evaluate the effect of other substituents at this position, we tested 4-amino-5-methyl-7-(2-*C*-methyl-β-

D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**31**). This methyl derivative displayed a weak inhibitory effect on HCV RNA replication (EC_{50} = 34 μM) and was not cytotoxic at up to 100 μM, as measured in the MTS assay. In light of the high potency of the other 5-substituted derivatives, we decided to evaluate the corresponding triphosphate as an inhibitor of HCV NS5B-mediated RNA synthesis. The triphosphate of **31** was found to be a potent inhibitor of RNA synthesis (IC_{50} = 0.57 μM). The effect of larger, electronegative substituents in the 5 position was further probed by the evaluation of the carbonitrile (**32**), the carboxamide (**33**), and the carboxylic acid (**34**). Compounds **32** and **33** gave rise to moderate to potent inhibition of HCV RNA replication (EC_{50} = 3.1 and 0.08 μM, respectively), whereas carboxylic acid **34** was significantly less potent in the cell-based assay (EC_{50} = 70 μM). Both **32** and **33**, however, displayed cytotoxicity ($CC_{50} \approx$ 50 and 33 μM, respectively) in the MTS assay.

The effect on activity of substituents at the pyrrolo[2,3-*d*]pyrimidine 2-position was investigated by the evaluation of 2,4-diaminopyrrolo[2,3-*d*]pyrimidine-2′-*C*-methylribonucleoside (**35**) and 4-amino-2-fluoro-pyrrolo[2,3-*d*]pyrimidine-2′-*C*-methylribonucleoside (**36**). Both compounds had minimal effect on reducing HCV RNA levels in the cell-based assay (EC_{50} > 100 μM).

The high potency of the 5-substituted 4-amino-pyrrolo[2,3-*d*]pyrimidines **25**−**27** and **32**−**33** led us to examine whether a similar type of substitution on guanosine analogue **20** would confer activity in the cell-based assay for HCV replication. Unfortunately, the evaluation of the 5-chloro derivative **37** revealed no significant effect on intracellular HCV RNA levels (EC_{50} > 100 μM).

Calculation of Conformational Preference for Ribonucleosides 9, 12, and 16. The conformational preferences of **9**, **12**, and **16** were calculated by molecular mechanics as described in the Experimental Section. The conformational analysis revealed no remarkable difference among the three compounds that might help explain the lack of potency of **12** and **16**. All were calculated to prefer a Northern, 3′-endo ribose conformation by 2.5−3.0 kcal/mol over the Southern, 2′-endo conformer as a result of the presence of the 2′-*C*-methyl group and to prefer an anti conformation of the base by a margin of 0.4−2.3 kcal/mol. Minor differences in the shape of the heterobases were observed as a result of the differences in the positioning of the endocyclic nitrogens, but the differences were too subtle to explain the dramatic differences in the potency of these ribonucleosides.

Evaluation of Possible Contacts between Substituents in the Pyrrolo[2,3-*d*]pyrimidine 5 Position and HCV NS5B. A model of nucleoside inhibitors in the HCV NS5B active site was created[7] from the HCV NS5B crystal structure[21] and the crystal structure of the φ6 RdRp with an initiation complex bound.[22] The steric and electrostatic environment of the inhibitor 5 position in the model was examined to help rationalize the SAR at this position. The closest polar functionality in the enzyme accessible from the inhibitor 5 position is the side-chain amino group of Lys141, at a distance of 4 Å. The examination of several overlaid HCV NS5B crystal structures from the PDB reveals that the Lys141 side chain has relatively high B factors and that its conformation differs in the various structures, suggesting a flexibility that might facilitate interaction with appropriate inhibitor functionality. Increased potency upon substitution with the electronegative fluorine (**25**) and with a hydrogen-bonding cyano (**32**) or carboxamide group (**33**) is consistent with direct interaction with Lys141. The carboxylic acid derivative **34**, which we hoped could form a salt bridge with the Lys141 basic side chain, unfortunately proved inactive. This inactivity, however, may be speculated to be the result of decreased cellular permeability due to the molecule's net charge under assay conditions.

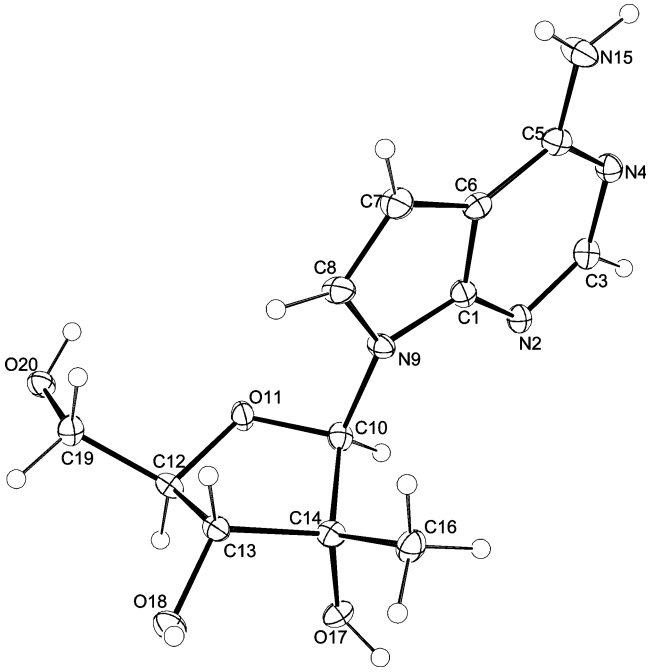


Figure 2. ORTEP perspective view of **9** showing the crystallographic numbering scheme. The thermal ellipsoids for the non-H atoms are drawn at the 50% level, and the H atoms are an arbitrary size.

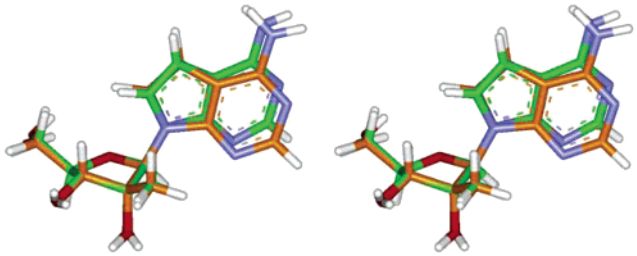


Figure 3. Stereoview of the crystallographically determined structure of **9** (green) and its global minimum conformation by molecular mechanics (orange).

Calculation of Conformational Preference and Solid-State Structure of 9. The solid-state structure of **9** (Figure 2) was determined by single-crystal X-ray crystallography. The conformational space of **9** was evaluated using molecular mechanics with the MMFFs force field and a dielectric constant of 50. A large number of conformations were generated using the JG distance geometry program and minimized with Batch-Min. The lowest-energy conformation found was overlaid with the crystallographically determined structure with respect to the furanose ring atoms (Figure 3). The RMSD for the ring atom positions was 0.017 Å, and the

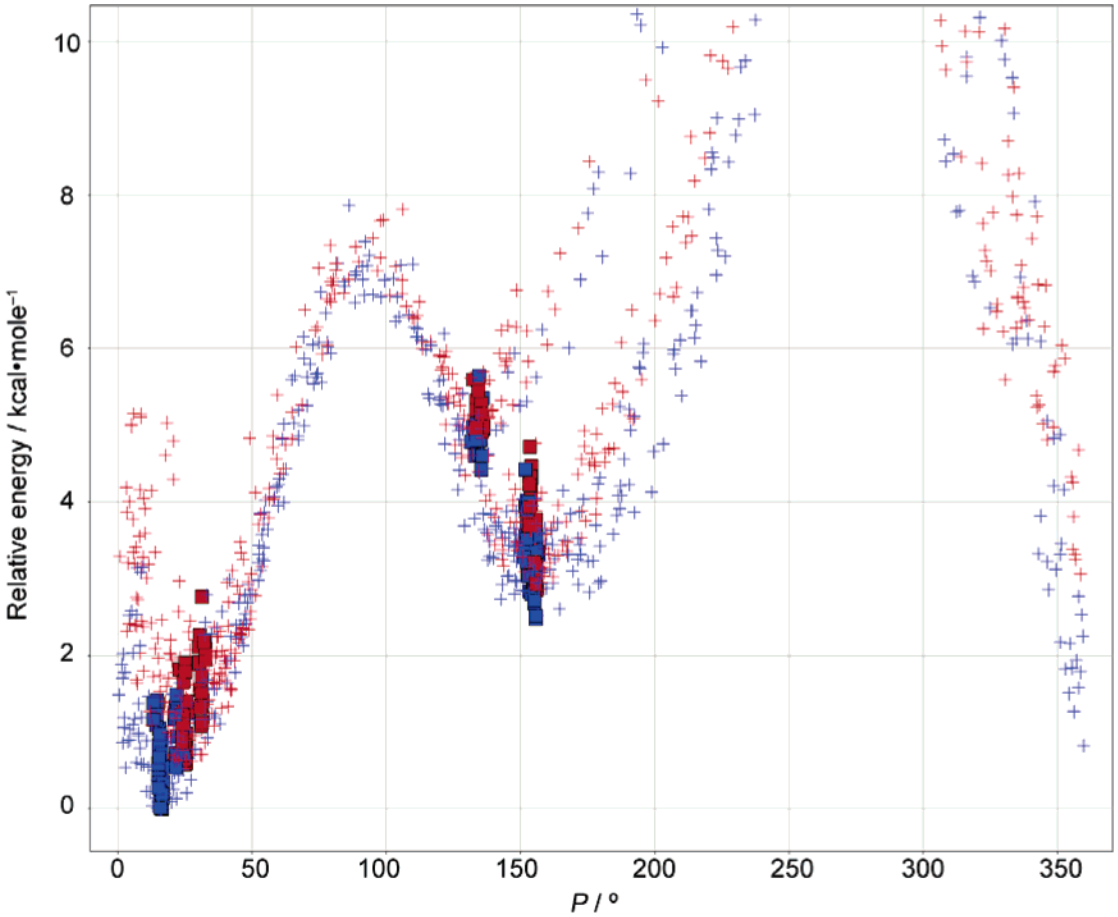


Figure 4. Plot of pseudorotational parameter *P* versus relative energy in kcal mol^{-1} for conformations of **9** calculated by full minimization (■) or minimization with the furanose ring conformation constrained (+). Heterobase orientation is indicated by color (blue = anti; red = syn).

Table 2. Phosphorolysis of the Glycosidic Bond of Adenosine, 2′-*C*-Methyladenosine (**1**), 4-Amino-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**9**), and 4-Amino-5-fluoro-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**25**) Catalyzed by Purine Nucleoside Phosphorylase (PNP)[a]

	% phosphorolysis by PNP		% conversion by ADA	
	1.8 U/mL	18 U/mL	0.00625 U/mL	1.25 U/mL
adenosine	100	100	96	100
1	19	100	0	100
9	0	0	0	0
25	0	0	0	0

[a] The results indicate that under the experimental conditions adenosine and **1** are substrates for PNP-mediated phosphorolysis, whereas no conversion of pyrrolo[2,3-*d*]pyrimidine derivatives **9** and **25** was detected. Adenosine deaminase-catalyzed conversion of adenosine, 2′-*C*-methyladenosine (**1**), 4-amino-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**9**), and 4-amino-5-fluoro-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**25**). Under the reaction conditions, adenosine and **1** are converted to inosine and 2′-*C*-methylinosine, respectively, whereas no conversion of pyrrolo[2,3-*d*]pyrimidine derivatives **9** and **25** was detected.

pseudorotational parameters *P* and *τ* were in close agreement (crystal: $P = 15.4°$, $\tau = 39.0°$; MMFFs: $P = 16.4°$, $\tau = 39.0°$). The orientation of hydroxyl groups and the 4-amino-7*H*-pyrrolo[2,3-*d*]pyrimidine base was also in agreement between the modeled and experimentally determined structures. Figure 4 depicts a plot of relative energy versus pseudorotational angle for the conformations determined by full minimization (■) and constrained minimization (+). On the basis of this analysis, the Northern conformation of **9** that was observed crystallographically is calculated to lie 2.5 kcal/mol below the lowest-energy Southern conformation; the anti base conformation is preferred to syn by 0.6 kcal/mol.

Table 3. Pharmacokinetic Parameters in Rat of Compound **25** Were Compared to That of 2′-*C*-Methyladenosine (**1**)

	1	**25**
Cl_p (mL/min/kg)	>200	44
$T_{1/2}$ (h)	0.3	5.1
C_{max} (*μ*M)	nd	0.6
T_{max} (h)	nd	0.5
F (%)	0	51

[a] Male CRL rats were dosed iv ($n = 2$) at 1.0 mg/kg and po at 2 mg/mL ($N = 3$).

Enzymatic Conversions Mediated by Purine Nucleoside Phosphorylase and Adenosine Deaminase. The ability of the two most potent NIs 4-amino-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**9**) and 4-amino-5-fluoro-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**25**) to function as substrates for PNP and ADA were examined (Table 2). The susceptibility of adenosine, and analogues **1**, **9**, and **25**, to cleavage by PNP, was determined at 1.8 and 18 U/mL and reaction times from 170 h (at 4−10 °C). The positive control for the assay, adenosine, was quantitatively cleaved while the otherwise unmodified 2′-*C*-methyladenosine (**1**) was only slowly converted. No appreciable conversion of the pyrrolo[2,3-*d*]pyrimidine derivatives **9** and **25** was observed (Table 2). Similarly,

we evaluated the ability of these compounds to function as substrates in the ADA-mediated conversion to the corresponding inosine derivatives at enzyme concentrations of 0.00625 and 1.25 U/mL. At the lower enzyme concentration, adenosine was converted to inosine, whereas 2′-*C*-methyladenosine (**1**) was not measurably affected. At higher enzyme concentrations, however, both adenosine and **1** were quantitatively converted to their corresponding inosine derivatives, whereas the pyrrolo[2,3-*d*]pyrimidine derivatives **9** and **25** were not substrates for this enzyme (Table 2).

Pharmacokinetic Studies. To evaluate the potential clinical utility of the 4-amino-7*H*-pyrrolo[2,3-*d*]-pyrimidine ribonucleosides, we compared the pharmacokinetic profile in the rat of **25** to that of 2′-*C*-methyladenosine **1** (Table 2). The oral bioavailablity of **25** was 51%, with a plasma clearance of 44 mL min^{-1} kg^{-1} and a half-life of 5.1 h. In comparison, plasma levels were below the level of detection post per oral dosing of **1** with clearance in excess of hepatic bloodflow (>200 mL min^{-1} kg^{-1}).[5]

Discussion

2′-*C*-Methyladenosine is a potent inhibitor of HCV RNA replication in vitro but is not orally bioavailable in rats.[5] We have suggested that the lack of oral bioavailability may be due in large part to the ability of 2′-*C*-methyladenosine to function as a substrate for PNP and ADA.[8] In light of these observations, we decided to examine 2′-*C*-methylribonucleosides containing heterobases with altered placement and number of non-hydrogen-bond-forming, endocyclic nitrogens for their ability to inhibit HCV RNA replication. This strategy for improving the activity and bioavailability was supported by previous studies, in which it was shown that nucleoside analogues containing 4-amino-pyrrolo[2,3-*d*]pyrimidine heterobases were inert to ADA-mediated hydrolysis.[23] We suspected that other metabolic enzymes that act on purine ribonucleosides might display similar sensitivity to subtle changes in the purine heterobase.

The replacement of the 6-aminopurine heterobase of the potent nucleoside inhibitor **1** with 4-amino-pyrrolo-[2,3-*d*]pyrimidine generated an equipotent inhibitor (**9**) of HCV RNA replication. However, two closely related ribonucleosides, in which 6-aminopurine was substituted with either 4-amino-pyrazolo[3,4-*d*]pyrimidine (**12**) or 4-amino-pyrrolo[3,2-*c*]pyridine (**16**), lacked any ability to attenuate HCV RNA levels. In an effort to better understand the inactivities of ribonucleosides **12** and **16**, we calculated the conformational preferences for both compounds, as well as for **9**. The calculated conformational preference of **9** was in agreement with the solid-state structure determined by single-crystal X-ray crystallography and is similar to the previously described conformational preference for the corresponding purine derivatives. The computations of conformational preference for **12** and **16** revealed very limited differences in the shape of the heterobases relative to **9**. These differences did not significantly affect the syn/anti position of the heterobase nor the north/south preference of the 2′-*C*-methylribose. Hence, our computations do not explain the observed inactivity of **12** and **16**.

The potency of 4-amino-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**9**) in the cell-based assay was not mirrored by the corresponding guanosine mimic, 2-amino-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (**20**), in the cell-based assay. Despite this dramatic difference, the triphosphates of both **9** and **20** were equally potent inhibitors of HCV NS5B-mediated RNA synthesis, suggesting that the lack of potency of **20** in the cell-based assay is not due to lack of recognition of the nucleoside triphosphate or lack of ability of the triphosphate to function as a "chain terminator" in the HCV NS5B-catalyzed polymerization reaction. In contrast, these data suggest that the inactivity of **20** is likely due to lack of cellular uptake and/or inefficient intracellular metabolism to the triphosphate. This conclusion is supported by experiments in which radiolabled compound was used to demonstrate that uptake and metabolism to the triphosphate in replicon-containing cells are inefficient for compound **20** (data not shown).

The similar potencies of 2′-*C*-methyladenosine (**1**) and its pyrrolo[2,3-*d*]pyrimidine analogue (**9**) indicated some tolerance for changes in the stereoelectronic features of the pyrrolo[2,3-*d*]pyrimidine 5 position (corresponding to the purine 7 position). The extent of this tolerance was further defined by the activities of the ribonucleoside derivatives substituted with fluoro (**25**), chloro (**26**), bromo (**27**), nitrile (**32**), and carbamoyl (**33**) in the pyrrolo[2,3-*d*]pyrimidine 5 position. The potency of the 5-halogen substituted 2′-*C*-methyl ribonucleosides declined with increasing atomic radius of the substituent, whereas the cytotoxicity was found to increase, leaving a narrow 100-fold window between activity and cytotoxicity for 5-bromo derivative (**27**). The high potency of the 5-fluoro derivative in the cell-based assay represents an approximate 4-fold improvement relative to the nonsubstituted derivative (**9**). In contrast, the intrinsic potency of **25** was slightly less than that of **9**. This apparent discrepancy was addressed by experiments in which a radiolabled compound was used to demonstrate a more efficient uptake and metabolism to the triphosphate in the replicon-containing cells for compound **25** as compared to those of **9** (data not shown). Despite this difference, cellular uptake and metabolism to their respective triphosphates were generally efficient for these adenosine analogues and, hence, in line with their relatively high potencies in the cell-based assay as compared to their intrinsic potencies. The addition of a carbamoyl substituent resulted in a compound that was about equipotent to the fluoro derivative but that displayed significant cytotoxicity. A similar increase in cytotoxicity was seen for the nitrile derivative. Notably, the 5-methyl substituted derivative (**31**) was weakly inhibitory on HCV RNA replication in the cell-based assay, whereas the corresponding triphosphate was a potent inhibitor of RNA synthesis by HCV NS5B. This pattern of activity is remarkably similar to that seen with guanosine mimic **20**. Thus, the presence of the nonpolar methyl substituent in the pyrrolo[2,3-*d*]pyrimidine 5 position appears to interfere with cellular uptake mechanisms and/or with the efficiency of intracellular conversion to the triphosphate. Likewise, the negative charge of the carboxylic acid derivative (**34**) may impede transport across the cell membrane and/

or interfere with enzymes involved in the requisite conversion to the triphosphate, giving rise to its poor activity in the HCV replicon assay.

The examination of the 5-substituted pyrrolo[2,3-*d*]-pyrimidine NIs in the model created from the crystal structure of HCV NS5B and a crystal structure of the φ6 RdRp bound to the φ6 initiation complex supports the observed activity of the fluorine-, nitrile-, and carbamoyl-substituted derivatives on the basis of a suggested interaction with Lys141. The model does not indicate a very tight environment around the pyrrolo-[2,3-*d*]pyrimidine 5 position and, hence, does not contradict the observed intrinsic potency of the triphosphate of 5-methyl derivative **31**.

The purine 2 position of adenosine is not utilized for Watson−Crick hydrogen-bond formation, and hence, we considered it possible that modification at this position may yield compounds with significant antiviral potency. However, even a very conservative change from hydrogen to fluoro at C2 on active nucleoside **9** rendered halogenated derivative **36** inactive in the replicon assay, suggesting that this position is not available for modification.

The findings presented here demonstrate that the 4-amino-pyrrolo[2,3-*d*]pyrimidine heterocycle is a good bioisosteric replacement for the endogenous adenine with respect to cell penetration, phosphorylation, and subsequent chain termination catalyzed by HCV NS5B polymerase. In contrast, the 4-amino-pyrazolo[3,4-*d*]-pyrimidine and 4-amino-pyrrolo[3,2-*c*]pyridine heterocycles are inefficient bioisosters for adenine. In addition, our observations reveal a remarkable intolerance of HCV NS5B to changes in the non-Watson−Crick hydrogen bond participating 2 and 6 positions of the 4-amino-pyrrolo[2,3-*d*]pyrimidine heterocycle. These results are in agreement with the results previously obtained in our laboratories, suggesting that changes in the 2 and 8 positions of 2′-*C*-methyladenosine did not generally lead to compounds that displayed significant inhibitory potency.[8] The substitution of the 4-amino-pyrrolo[2,3-*d*]pyrimidine 5 position with polar substituents is tolerated and leads to increased potency when the substituents are small and polar/electronegative. Furthermore, the inactivity of the 2-amino-5-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one derivative (**37**) shows that the inhibitory activities of the 5-substituted 4-amino-pyrrolo[2,3-*d*]-pyrimidine derivatives on HCV RNA replication do not generally extend to the 2-amino-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one series. With respect to the potency of the 4-amino-pyrrolo[2,3-*d*]pyrimidine derivatives, 4-amino-5-fluoro-pyrrolo[2,3-*d*]pyrimidine appears to be an especially effective bioisosteric replacement for adenine. The selectivity indexes for 4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**9**) and 4-amino-5-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**25**) are both >800-fold. Most importantly, bioisosteric replacement of adenine with 4-amino-pyrrolo[2,3-*d*]pyrimidine or 4-amino-5-fluoro-pyrrolo[2,3-*d*]pyrimidine abolishes the ability of the resulting 2′-*C*-methyl ribonucleosides to function as substrates for metabolic conversions mediated by ADA and PNP. The differences in susceptibility to enzymatic conversions lead to improved pharmacokinetic profiles, as illustrated by the oral bioavailability of the fluorinated derivative **25** in the rat. Hence, in contrast to 2′-*C*-methyladenosine, the 4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine inhibitor class displays pharmacokinetic profiles consistent with clinical utility.

Experimental Section

General Procedures. 4-Chloro-1*H*-pyrrolo[2,3-*d*]pyrimidine,[14] 2-amino-4-chloro-1*H*-pyrrolo[2,3-*d*]pyrimidine,[24] 2,4-dichloro-1*H*-pyrrolo[3,2-*c*]pyridine,[15] and 4-chloro-5-methyl-1*H*-pyrrolo[2,3-*d*]pyrimidine[19] were synthesized according to existing literature procedures. 4-Amino-1*H*-pyrazolo[3,4-*d*]pyrimidine (purchased from Aldrich A7,780-6) and 3,5-bis-*O*-(2,4-dichlorophenylmethyl)-1-*O*-methyl-α-D-ribofuranose (Lipomed NUC-55) were used as received. TLC was performed on silica 60 (Merck 5554 aluminum sheet), column chromatograpy on silica 60 (230−400-mesh ASTM) (Merck 9385). Molecular sieves (4 Å, Mallinckrodt 4494) were activated prior to use (120 °C in vacuo). ^{1}H and ^{13}C NMR spectra were obtained at 200 MHz (Varian Mercury VX) in 5-mm tubes unless otherwise indicated; chemical shifts are positive in the low-field direction. FAB mass spectra were recorded on a JEOL Hx110/110 mass spectrometer. High-resolution FAB spectra were obtained from UC Berkeley. All nucleosides screened in the cell-based assay were purified by preparative RP HPLC using a reverse-phase column (Phenomenex, Luna 10 μm, 21.2 mm × 250 mm, A = water, B = acetonitrile, 2 to 95% B in 60 min, flow 3 mL min^{-1}). Nucleosides were analyzed for purity by analytical RP HPLC (Phenominex C18 aqua 5 μm, 150 mm × 4.6-mm column A = 100 mM triethylammoniumacetate, pH 7, B = 10% 100 mM triethylammoniumacetate in acetonitrile, 100% B in 25 min., flow 1 mL min^{-1}). The purity of the nucleosides screened was generally greater than 98%.

Assay for Inhibition of HCV RNA Replication in Cells. Inhibition of HCV RNA replication was assayed in a subgenomic bicistronic replicon assay in HB-1 cells by in situ RPA as previously described.[5] The replicon EC_{50} values are the average of at least two separate potency determinations at 24 h (Table 1).

Assay for Inhibition of NS5B-Mediated RNA Synthesis in Vitro. Inhibition of the enzymatic activity of HCV NS5BΔ21 by the corresponding nucleoside triphosphates was determined as previously described.[5] The IC_{50} values for all active compounds are the averages of at least three separate determinations.

Assay for PNP-Mediated Phosphorolysis. Assay reactions (100 mM HEPES pH 7.0, 50 mM sodium phosphate pH 7.0, 200 μM nucleoside) were initiated with addition of 1.8 or 18 U/mL PNP (Sigma N-3514, isolated from human blood). The reactions were incubated at 4 °C for 170 h. The RP-HPLC analysis utilized a C18 column (Vydac C218TP, 150 mm × 4.6 mm, 5 μM) and guard column (Perkin-Elmer 0711-0092, 15 mm × 3.2 mm, 7 μM) with 10 mM potassium phosphate, 2 mM TBA (buffer A) and 50% methanol, 10 mM potassium phosphate, 2 mM TBA (buffer B), 1 mL/min flow rate. A 50-μL reaction volume injection and elution over the gradient yielded quantifiable nucleoside peaks (1−5% B over 15 min; 5−75% B over 2.5 min; hold 75% for 1 min), monitoring at 260 nm.

Assay for ADA-Mediated Conversion. Assay reactions included 5 mM Tris-HCl pH 7.4, 5 mM potassium phosphate, and 0.00625 or 1.25 U/mL adenosine deaminase (ADA, Sigma A-5168, type IX from calf spleen). The reactions were initiated with addition of 25 μM nucleoside. The reactions were incubated at room temperature for 30 min (0.00625 U/mL ADA) or 24 h (1.25 U/mL ADA) before the enzyme was heat inactivated (75 °C for 15 min). The RP-HPLC analysis utilized a SUPELCOSIL LC-18-S column (Supelco 58931, 150 mm × 4.6 mm, 5 μM) with 97.5%/2.5% 50 mM potassium phosphate pH 4.4/methanol (buffer A) and 80%/20% 50 mM potassium phosphate pH 4.4/methanol (buffer B), 1 mL/min flow rate. A 250-μL reaction volume injection and elution over the gradient

yielded quantifiable nucleoside peaks (0% B hold for 3 min, 0−100% B over 9 min, 100% hold for 3 min, 100% to 60% over 4.5 min) monitoring at 254 and 280 nm. Injections of adenosine and inosine yielded linear standard curves allowing for the quantitation of substrate utilization and product formation.

Pharmacokinetic Studies. To evaluate the potential clinical utility of the 4-amino-*7H*-pyrrolo[2,3-*d*]pyrimidine ribonucleosides, the pharmacokinetic profile in the rat of the most potent compound, **25**, was compared to that of 2′-*C*-methyladenosine **1** (Table 2). Male CRL rats were dosed iv ($N = 2$) at 1.0 mg/kg and po at 2 mg/mL ($N = 3$). Plasma samples were obtained and analyzed as previously described.[7]

Calculations. Nucleoside conformational preferences were calculated by molecular mechanics using the MMFFs force field and a dielectric constant of 50. For each nucleoside, 1000 conformers were generated using the JG distance geometry program.[25] and were minimized to low gradient using BatchMin.[26] To estimate the barrier to interconversion between the Northern and Southern conformers and the steepness of the minima, the conformers generated by distance geometry were also subjected to constrained minimization, holding one of the ribose ring dihedral angles at a random value between −42° and +42° by means of a harmonic force constant of 1000 kJ mol^{-1} rad^{-2}.

Solid-State Structure of 9. The structure of compound **9**, $C_{12}H_{16}N_4O_4$, has been determined by single-crystal X-ray crystallography. Diffraction-quality crystals were grown by the slow evaporation of a CH_3NO_2/CH_3CH_2OH solution of the compound, and the crystals that were obtained are orthorhombic with space group $P2_12_12$ and cell constants of $a = 16.330(6)$ Å, $b = 8.353(6)$ Å, $c = 8.78(1)$ Å, $V = 1198(2)$ Å^3, and $Z = 4$. The calculated density is 1.555 g cm^{-3}.

All diffraction measurements were made using monochromatized Mo Kα radiation ($\lambda = 0.71073$ Å) on a CCD area-detector equipped diffractometer, at $T = 100$ K, to a θ limit of 26.48°. There are 2472 unique reflections out of 12 981 measured, with 2127 observed at the $I \geq 2\sigma(I)$ level. The structure was solved by direct methods and was refined using full-matrix least-squares on F^2 using 185 parameters and all unique reflections. The refinement converged with agreement statistics of $R = 0.035$, $wR = 0.080$, $S = 1.01$, $(\Delta/\sigma)_{max} \leq 0.01$.

A computer-generated perspective view of the molecule is shown in Figure 2. Tables of the atomic parameters for the model along with interatomic distances and angles are available in the Supporting Information. The atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre (deposition no. 245444) and can be obtained, on request, from data_request@ccdc.cam.ac.uk or Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K.

Synthesis, Purification, and QC of Nucleoside Triphosphates. Nucleoside triphosphates were synthesized as previously described.[8] Purification was performed by anion exchange chromatography, and appropriate fractions were subsequently desalted by reverse-phase HPLC as described.[8] Purity was determined by analytical reverse-phase HPLC and anion exhange HPLC. The identities of the nucleoside triphosphates were confirmed by mass spectrometry as described.[8] The yield of purified nucleoside triphosphate following this procedure ranged from 5 to 15 mg. Purity of >90% was generally attained.

3,5-Bis-*O*-(2,4-dichlorophenylmethyl)-1-*O*-methyl-α-D-*erythro*-pentofuranos-2-ulose (4). To an precooled (0 °C) suspension of Dess−Martin periodinane (50.0 g, 118 mmol) in dichloromethane (350 mL) was added a solution of 3,5-bis-*O*-(2,4-dichlorophenylmethyl)-1-*O*-methyl-α-D-ribofuranose (**3**) (36.2 g, 75 mmol) in dichloromethane (200 mL) dropwise over 0.5 h. The reaction mixture was stirred at 0 °C for 0.5 h and then at room temperature for 3 days. The mixture was diluted with diethyl ether (600 mL) and poured into a precooled (0 °C) mixture of $Na_2S_2O_3 \cdot 5H_2O$ (180 g) in saturated aqueous sodium hydrogencarbonate (1400 mL). The layers were separated, and the organic layer was washed with saturated aqueous sodium hydrogencarbonate (600 mL), water (800 mL), and brine (600 mL), dried over $MgSO_4$, filtered, and evaporated in vacuo to give the title compound (34.2 g, 95%) as a colorless oil, which was used without further purification in the next step. ^{1}H NMR ($CDCl_3$): δ 3.50 (s, 3H), 3.79 (dd, $J = 11.3$ Hz, 3.5 Hz, 1H), 3.94 (dd, $J = 11.3$ Hz, 2.3 Hz, 1H), 4.20 (dd, $J = 1.3$ Hz, 8.4 Hz, 1H), 4.37 (ddd, $J = 8.4$ Hz, 3.5 Hz, 2.3 Hz, 1H), 4.58, 4.69 (2d, $J = 13.0$ Hz, 2H), 4.87 (d, $J = 1.3$ Hz, 1H), 4.78, 5.03 (2d, $J = 12.5$ Hz, 2H), 7.19−7.26, 7.31−7.42 (2m, 6H).

3,5-Bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-1-*O*-methyl-α-D-ribofuranose (5). To a solution of methylmagnesium bromide in diethyl ether (0.48 M, 300 mL) at −55 °C was added dropwise a solution of 3,5-bis-*O*-(2,4-dichlorophenylmethyl)-1-*O*-methyl-α-D-*erythro*-pentofuranos-2-ulose (**4**) (17.40 g, 36.2 mmol) in diethyl ether (125 mL). The reaction mixture was allowed to warm to −30 °C and was stirred for 7 h at −30 to −15 °C, then poured into ice-cold water (500 mL), and the mixture vigorously stirred at room temperature for 0.5 h. The mixture was filtered through a Celite pad which was thoroughly washed with diethyl ether. The organic layer was dried ($MgSO_4$), filtered, and evaporated in vacuo. The residue was purified on silica gel using hexanes and hexanes/ethyl acetate (9:1) as the eluent to give the title compound (16.7 g, 93%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 1.36 (d, $J = 0.9$ Hz, 3H), 3.33 (q, $J = 0.9$ Hz, 1H), 3.41 (d,, $J = 3.3$ Hz, 1H), 3.46 (s, 3H), 3.66 (d, $J = 3.7$ Hz, 2H), 4.18 (apparent q, 1H), 4.52 (s, 1H), 4.60 (s, 2H), 4.63, 4.81 (2d, $J = 13.2$ Hz, 2H), 7.19−7.26, 7.34−7.43 (2m, 6H). HRMS FAB: calcd for $C_{21}H_{21}Cl_4O_4 + Li^+$ 501.0381, found 501.0378.

4-Chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (7). To a solution of 3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-1-*O*-methyl-α-D-ribofuranose (**5**) (9.42 g, 19 mmol) in anhydrous dichloromethane (285 mL) at 0 °C was added HBr (5.7 M in acetic acid, 20 mL, 114 mmol) dropwise. The resulting solution was stirred at 0 °C for 1 h and then at room temperature for 3 h, evaporated in vacuo, and coevaporated with anhydrous toluene (3 × 40 mL). The oily residue was dissolved in anhydrous acetonitrile (50 mL) and added to a solution of the sodium salt of 4-chloro-1*H*-pyrrolo[2,3-*d*]pyrimidine (**6**) in acetonitrile (generated in situ from 4-chloro-1*H*-pyrrolo[2,3-*d*]pyrimidine (8.76 g, 57 mmol) in anhydrous acetonitrile (1000 mL), and NaH (60% in mineral oil, 2.28 g, 57 mmol), after 4 h of vigorous stirring at room temperature). The combined mixture was stirred at room temperature for 1 day and then evaporated to dryness. The residue was suspended in water (250 mL) and extracted with ethyl acetate (2 × 500 mL). The combined extracts were washed with brine (300 mL), dried over Na_2SO_4, filtered, and evaporated. The crude product was purified on silica gel using ethyl acetate/hexane (1:3 and 1:2) as the eluent. Fractions containing the product were combined and evaporated in vacuo to give the desired product (5.05 g, 43%) as colorless foam. ^{1}H NMR ($CDCl_3$): δ 0.93 (s, 3H), 3.09 (s, 1H), 3.78 (dd, $J = 10.9$ Hz, 2.5 Hz, 1H), 3.99 (dd, $J = 10.9$ Hz, 2.2 Hz, 1H), 4.23−4.34 (m, 2H), 4.63, 4.70 (2d, $J = 12.7$ Hz, 2H), 4.71, 4.80 (2d, $J = 12.1$ Hz, 2H), 6.41 (s, 1H), 6.54 (d, $J = 3.8$ Hz, 1H), 7.23−7.44 (m, 6H), 7.69 (d, $J = 3.8$ Hz, 1H), 8.64 (s, 1H). HRMS FAB: calcd for $C_{26}H_{22}Cl_5N_3O_4 + H^+$ 616.0131, found 616.0118.

4-Chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo-[2,3-*d*]pyrimidine (8). To a solution of 4-chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (**7**) (5.42 g, 8.8 mmol) in dichloromethane (175 mL) at −78 °C was added boron trichloride (1 M in dichloromethane, 88 mL, 88 mmol) dropwise. The mixture was stirred at −78 °C for 2.5 h, and then at −30 to −20 °C for 3 h. The reaction was quenched by addition of methanol/dichloromethane (1:1) (90 mL), and the resulting mixture was stirred at −15 °C for 30 min, then neutralized with aqueous ammonia at 0 °C, and stirred at room temperature for 15 min. The solid was filtered and washed with dichloromethane/methanol (1:1, 250 mL). The combined filtrate was evaporated, and the residue was purified by flash chromatography on silica gel using dichloromethane and a dichloromethane/methanol

(99:1, 98:2, 95:5, and 90:10) gradient as the eluent to furnish the desired compound (1.73 g, 66%) as colorless foam, which turned into an amorphous solid after treatment with acetonitrile. ^{1}H NMR (DMSO-d_6): δ 0.64 (s, 3H), 3.61−3.71 (m, 1H), 3.79−3.88 (m, 1H), 3.89−4.01 (m, 2H), 5.15−5.23 (m, 3H), 6.24 (s, 1H), 6.72 (d, J = 3.8 Hz, 1H), 8.13 (d, J = 3.8 Hz, 1H), 8.65 (s, 1H). HRMS FAB: calcd for $C_{13}H_{18}N_4O_4 + H^+$ 300.0751, found 300.0736.

4-Amino-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo-[2,3-*d*]pyrimidine (9). To 4-chloro-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**8**) (1.54 g, 5.1 mmol) was added methanolic ammonia (saturated at 0 °C) (150 mL). The mixture was heated in a stainless steel autoclave at 85 °C for 14 h and then cooled and evaporated in vacuo. The crude mixture was purified on silica gel using dichloromethane/methanol (9:1) as the eluent to give the title compound as a colorless foam (0.80 g, 56%), which separated as an amorphous solid after treatment with acetonitrile. The amorphous solid was recrystallized from methanol/acetonitrile; mp 222 °C. ^{1}H NMR (DMSO-d_6): δ 0.62 (s, 3H), 3.57−3.67 (m, 1H), 3.75−3.97 (m, 3H), 5.00 (s, 1H), 5.04 (d, J = 6.8 Hz, 1H), 5.06 (t, J = 5.1 Hz, 1H), 6.11 (s, 1H), 6.54 (d, J = 3.6 Hz, 1H), 6.97 (br s, 2H), 7.44 (d, J = 3.6 Hz, 1H), 8.02 (s, 1H). HRMS FAB: calcd for $C_{12}H_{16}N_4O_4 + H^+$ 281.1250, found 281.1243.

4-Amino-1-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-*β*-D-ribofuranosyl]-1*H*-pyrazolo[3,4-*d*]pyrimidine (11). 3,5-Bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-1-*O*-methyl-α-D-ribofuranose (**5**) (1.00 g, 2.02 mmol) was dissolved in dichloromethane (20 mL), and HBr gas was bubbled in the solution for 5 min until it was saturated. The resulting solution was stirred at room temperature for 10 min, evaporated in vacuo, and coevaporated with anhydrous toluene (10 mL). To this compound was added the sodium salt of 4-amino-1*H*-pyrazolo[3,4-*d*]pyrimidine (**10**) in 1-methyl-2-pyrrolidinone (generated in situ from 4-amino-1*H*-pyrazolo[3,4-*d*]pyrimidine (0.43 g, 3.18 mmol) in anhydrous 1-methyl-2-pyrrolidinone (10 mL) and NaH (60% in mineral oil, 150 mg, 3.8 mmol) after 30 min of vigorous stirring at room temperature). The resulting solution was stirred overnight at room temperature. The mixture was diluted with toluene (50 mL), washed with brine (3 × 50 mL), and concentrated in vacuo. The residue was purified on silica gel using ethyl acetate as the eluent. Fractions containing the product were pooled and evaporated in vacuo to give the desired compound (400 mg, 33%) as a colorless solid. ^{1}H NMR (DMSO-d_6): δ 0.97 (s, 3H), 3.77 (m, 2H), 4.23 (m, 1H), 4.42 (m, 1H), 4.49 (s, 2H), 4.65 (m, 2H), 5.48 (s, 1H), 6.14 (s, 1H), 7.13−7.63 (m, 6H), 7.78 (br s, 2H), 8.19 (s, 1H), 8.20 (s, 1H). HRMS FAB: calcd. for $C_{25}H_{23}Cl_4N_5O_4$ + H 598.0582, found 598.0581.

4-Amino-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-1*H*-pyrazolo-[3,4-*d*]pyrimidine (12). To a solution of 4-amino-1-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-*β*-D-ribofuranosyl]-1*H*-pyrazolo[3,4-*d*]pyrimidine (**11**) (0.20 g, 0.33 mmol) in dichloromethane (10 mL) at −78 °C was added boron trichloride (1 M in dichloromethane) (3.0 mL, 3.0 mmol) dropwise. The mixture was stirred at −78 °C for 0.5 h and then at −45 to −30 °C for 2 h. The reaction was quenched by addition of sodium acetate (1.0 g) and methanol (10 mL). The solution was evaporated in vacuo, and the residue was purified by flash chromatography over silica gel using dichloromethane and dichloromethane/methanol (95:5 to 90:10) as the eluents to furnish the desired compound (60 mg, 64%) as slightly tan solid. This compound was recrystallized from methanol and acetonitrile to give the desired compound (40 mg, 41%) as an off-white solid. ^{1}H NMR (DMSO-d_6): δ 0.75 (s, 3H), 3.59 (m, 1H), 3.69 (m, 1H), 3.91 (m, 1H), 4.12 (m, 1H), 4.69 (t, J = 5.1 Hz, 1H), 5.15 (m, 2H), 6.13 (s, 1H), 7.68−7.96 (br, 2H,), 8.18 (s, 1H), 8.21 (s, 1H). HRMS FAB: calcd for $C_{11}H_{15}N_5O_4$ + H 282.1202, found 282.1203.

4,6-Dichloro-1-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-*β*-D-ribofuranosyl]-1*H*-pyrrolo[3,2-*c*]pyridine (14). Through a solution of 3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-1-*O*-methyl-α-D-ribofuranose (**5**) (2.00 g, 4.04 mmol) in dichloromethane (50 mL) was bubbled HBr until the solution was saturated (5 min). The resulting solution was stirred at 0 °C for 30 min, evaporated in vacuo, and coevaporated with anhydrous toluene (20 mL). To this compound was added the sodium salt of 4,6-dichloro-1*H*-pyrrolo[3,2-*c*]pyridine (**13**) in 1-methyl-2-pyrrolidinone (generated in situ from 4,6-dichloro-1*H*-pyrrolo[3,2-*c*]pyridine (1.13 g, 6.04 mmol) in anhydrous 1-methyl-2-pyrrolidinone (50 mL) and NaH (60%, 0.26 g, 6.50 mmol) after 1 h of vigorous stirring at room temperature). The resulting solution was stirred overnight at room temperature. The mixture was diluted with toluene (200 mL) and filtered to remove any solid. The filtrate was washed with brine (3 × 100 mL) and was then concentrated under reduced pressure. The residue was purified on silica gel using ethyl acetate/hexane (20:80) to give the desired compound (0.98 g, 37%) as a colorless solid. ^{1}H NMR ($CDCl_3$): δ 0.85 (s, 3H), 3.14 (s, 1H), 3.76 (m, 1H), 3.95 (m, 1H), 4.22 (m, 1H), 4.60−4.76 (m, 5H), 5.85 (s, 1H), 6.58 (d, J = 3.4 Hz, 1H), 7.22−7.45 (m, 7H), 7.54 (d, J = 3.5 Hz, 1H). HRMS: calcd for $C_{27}H_{22}Cl_6N_2O_4$ + H 648.9787, found 648.9783.

4-Amino-6-chloro-1-(2-*C*-methyl-*β*-D-ribofuranosyl)-1*H*-pyrrolo[3,2-*c*]pyridine (15). To 4,6-dichloro-1-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-*β*-D-ribofuranosyl]-1*H*-pyrrolo[3,2-*c*]pyridine (**14**) (0.65 g, 1.0 mmol) in dichloromethane (10 mL) at −78 °C was added boron trichloride (1 M in dichloromethane, 10 mL, 10 mmol) dropwise. The mixture was stirred at −78 °C for 0.5 h and then at −45 to −30 °C for 2 h. The reaction was quenched by addition of sodium acetate (3.0 g) and methanol (20 mL). The solution was evaporated, and the residue was purified by flash chromatography over silica gel using dichloromethane and then methanol/dichloromethane (5:95) as the eluents to furnish the desired compound (200 mg, 64%) as slightly tan solid. The above solid was heated with liquid ammonia (ca. 10 mL) and CuI (0.1 g) at 130 °C for 24 h. The mixture was evaporated, and the residue was purified by column chromatography on silica gel. The desired product was eluted with methanol/dichloromethane (5:95 to 10:90) to afford a colorless solid (120 mg, 38% for the two steps). ^{1}H NMR (DMSO-d_6): δ 0.60 (s, 3H), 3.65 (m, 1H), 3.78 (m, 3H), 5.00 (d, J = 6.1 Hz, 1H), 5.08 (t, J = 4.9 Hz, 1H), 5.19 (s, 1H), 5.73 (s, 1H), 6.55 (s, 2H), 6.63 (d, J = 3.0 Hz, 1H), 6.82 (s, 1H), 7.49 (d, J = 3.4 1H). HRMS FAB: calcd for $C_{13}H_{16}ClN_3O_4$ + H 314.0908, found 314.0902.

4-Amino-1-(2-*C*-methyl-*β*-D-ribofuranosyl)-1*H*-pyrrolo-[3,2-*c*]pyridine (16). 4-Amino-6-chloro-1-(2-*C*-methyl-*β*-D-ribofuranosyl)-1*H*-pyrrolo[3,2-*c*]pyridine (**15**) (40 mg, 0.13 mmol), concentrated aqueous ammonia (0.5 mL), and Pd/C (10%, 20 mg) in methanol (5 mL) were stirred under a normal atmosphere of hydrogen overnight. The mixture was filtered and evaporated. The residue was purified by preparative reverse-phase HPLC using a linear gradient of acetonitrile−water (1:99−15:85 in 15 min, C18 column, 9 mL/min) to give a colorless solid (20 mg, 56%). ^{1}H NMR (DMSO-d_6): δ 0.62 (s, 3H), 3.67 (m, 1H), 3.88 (m, 3H), 5.16 (m, 2H), 5.34 (s, 1H), 5.95 (s, 1H), 7.06 (d, J = 3.2 Hz, 1H), 7.25 (d, J = 7.1 Hz, 1H), 7.60 (d, J = 7.1 Hz, 1H), 7.85 (d, J = 3.4 Hz, 1H). 8.15 (s, 2H). HRMS FAB: calcd for $C_{13}H_{17}N_3O_4$ + H 280.1297, found 280.1290.

2-Amino-4-chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (18). To a precooled (0 °C) 3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-1-*O*-methyl-α-D-ribofuranose (**5**) (1.27 g, 2.57 mmol) in dichloromethane (30 mL) was added HBr (5.7 M in acetic acid; 3 mL) dropwise. The reaction mixture was stirred at room temperature for 2 h, concentrated in vacuo, and coevaporated with toluene (2 × 15 mL). The resulting oil was dissolved in acetonitrile (15 mL) and added dropwise into a well-stirred mixture of 2-amino-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (433 mg, 2.57 mmol), KOH (85%, powdered) (0.51 g, 7.7 mmol), and tris[2-(2-methoxyethoxy)-ethyl]amine (165 μL, 0.51 mmol) in acetonitrile (30 mL). The resulting mixture was stirred at room temperature for 1 h, filtered, and evaporated in vacuo. The residue was purified on a silica gel column using hexanes/ethyl acetate (5:1, 3:1, and 2:1) as the eluent to give the title compound as a colorless

foam (0.65 g, 40%). ^{1}H NMR ($CDCl_3$): δ 0.97 (s, 3H 3.11 (br s, 1H), 3.74 (dd, J = 11.0 Hz, 2.5 Hz, 1H), 3.95 (dd, J = 11.0 Hz, 1.6 Hz, 1H), 4.25−4.19 (m, 2H), 4.56−4.82 (4d, 4H), 4.98 (br s, 2H), 6.21 (s, 1H), 6.31 (d, J 3.8 Hz, 1H), 7.22−4.43 (m, 7H). HRMS: calcd for $C_{26}H_{23}Cl_5N_4O_4 + H^+$ 631.0240, found 631.0231.

2-Amino-4-chloro-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (19). To a solution of 2-amino-4-chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**18**) (630 mg, 1.0 mmol) in dichloromethane (20 mL) at −78 °C was added boron trichloride (1 M in dichloromethane) (10 mL, 10 mmol). The mixture was stirred at −78 °C for 2 h and then at −20 °C for 2.5 h. The reaction was quenched with dichloromethane/methanol (1:1) (10 mL), stirred at −20 °C for 0.5 h, and neutralized at 0 °C with aqueous ammonia. The solid was filtered and then washed with dichloromethane/methanol (1:1), and the combined filtrate was evaporated in vacuo. The residue was purified on silica gel using dichloromethane/methanol (50:1 and 20:1) as the eluent to give the title compound (250 mg, 84%) as a colorless foam. ^{1}H NMR (methanol-d_4) δ: 0.86 (s, 3H), 3.81 (m, 1H), 3.93−4.10 (3m, 3H), 6.16 (s, 1H), 6.38 (d, J = 3.7 Hz, 1H), 7.43 (d, J = 3.7, 1H). HRMS: calcd for $C_{12}H_{15}ClN_4O_4 + H^+$ 315.0860, found 315.0856.

2-Amino-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo-[2,3-*d*]pyrimidin-4(3*H*)-one (20). A mixture of 2-amino-4-chloro-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**19**) (90 mg, 0.3 mmol) in aqueous NaOH (2 N, 9 mL) was heated at reflux temperature for 5 h and then neutralized at 0 °C with 2 N aqueous HCl and evaporated to dryness. Purification on silica gel using dichloromethane/methanol (5:1) as the eluent yielded the title compound (70 mg, 82%) as a white solid. ^{1}H NMR (methanol-d_4): δ 0.86 (s, 3H), 3.79 (m 1H), 3.90−4.05 (m, 3H), 6.06 (s, 1H), 6.42 (d, J = 3.7 Hz, 1H), 7.05 (d, J = 3.7 Hz, 1H). HRMS: calcd for $C_{12}H_{16}N_4O_5 + H^+$ 297.1199, found 297.1200.

4-Chloro-5-fluoro-7*H*-pyrrolo[2,3-*d*]pyrimidine (23). To a solution of 4-chloro-5-bromo-7*H*-pyrrolo[2,3-*d*]pyrimidine (**21**) (0.92 g, 4.0 mmol) in tetrahydrofuran (25 mL) was added *n*-BuLi (2.5 M solution in hexane, 3.48 mL) dropwise at −78 °C, and the reaction mixture was stirred at −78 °C for an additional 30 min. To this solution was added trimethylstannyl chloride (0.88 g, 4.4 mmol) in tetrahydrofuran (8 mL) dropwise over a period of 10 min. The reaction mixture was allowed to warm to room temperature and was stirred overnight. Saturated aqueous ammonium chloride (60 mL) was added and extracted with ethyl acetate (3 × 70 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, and evaporated to dryness. The residue was purified over silica gel to give the desired stannane (0.80 g, 63%) as a colorless solid. To a solution of this compound (1.97 g, 6.20 mmol) in acetonitrile (60 mL) was added 1-(chloromethyl)-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate) (2.40 g, 6.5 mmol) in one portion, and the reaction mixture was stirred at room temperature for 7 h. The precipitate was filtered off, and the filtrate was evaporated in vacuo. The residue was purified on silica gel using ethyl acetate/hexane (3:7) as the eluent to give the title compound (0.22 g, 21%) as a colorless solid. ^{1}H NMR (methanol-d_4): δ 8.53 (s, 1H), 7.37 (d, J = 2.8 Hz, 1H). ^{19}F NMR (DMSO-d_6): δ −171.5. HRMS FAB: calcd for $C_6H_3ClFN_3 + H^+$ 172.0078, found 172.0079.

4-Amino-5-fluoro-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (25). To a solution of **23** (0.075 g, 0.44 mmol), 2,3,5-tri-*O*-benzoyl-2-*C*-methyl-D-ribofuranose (**24**)[18] (0.25 g, 0.53 mmol) and triphenylphosphine (0.23 g, 0.88 mmol) in tetrahydrofuran (15 mL) was added diethyl azodicarboxylate (DEAD) (0.14 mL, 0.88 mmol). The reaction mixture was stirred at room temperature overnight. The solution was directly adsorbed onto silica gel and was purified over silica gel using ethyl acetate/hexane (1:9) as the eluent. Appropriate fractions were combined and evaporated. The residue was dissolved in dioxane (3 mL) and liquid ammonia (4 mL), and the mixture was heated in a steel bomb at 85 °C for 24 h. The solvent was evaporated, and the residue was purified over silica gel using methanol/dichloromethane (1:9) as the eluent to give the title compound (0.008 g, 5%). ^{1}H NMR (methanol-d_4): δ 8.07 (s, 1H), 7.41 (d, J = 2.2 Hz, 1H), 6.25 (d, J = 1.8 Hz), 4.09−3.95 (m, 3H), 3.82 (dd, J = 2.7, 12.5 Hz, 1H). ^{19}F NMR (methanol-d_4): δ −170.4. HRMS FAB: calcd for $C_{12}H_{15}FN_4O_4 + H^+$ 299.1156, found 299.1150.

4-Amino-5-chloro-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (26). To a precooled solution (0 °C) of **9** (50 mg, 0.18 mmol) in dimethylformamide (0.5 mL) was added *N*-chlorosuccinimide (0.024 g, 0.18 mmol) in dimethylformamide (0.5 mL) dropwise. The solution was allowed to warm to room temperature and was stirred for 2 h. The reaction was quenched with methanol (4 mL) and was evaporated in vacuo. The crude product was purified on silica gel using methanol/dichloromethane (1:9) as the eluent to give the desired product (27 mg, 48%) as a colorless solid. ^{1}H NMR (acetonitrile-d_3): δ 0.80 (s, 3H), 3.65−4.14 (overlapping m, 7H), 5.97 (br s, 2H), 6.17 (s, 1H), 7.51 (s, 1H), 8.16 (s, 1H). HRMS: calcd for $C_{12}H_{15}ClN_4O_4 + H^+$ 315.0860, found 315.0860.

4-Amino-5-bromo-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (27). To a precooled solution (0 °C) of **9** (50 mg, 0.18 mmol) in dimethylformamide (0.5 mL) was added *N*-bromosuccinimide (0.032 g, 0.18 mmol) in dimethylformamide (0.5 mL) dropwise. The solution was stirred at 0 °C for 20 min and then at room temperature for 10 min. The reaction was quenched by addition of methanol (4 mL) and was evaporated in vacuo. The crude product was purified on silica gel using methanol/dichloromethane (1:9) as the eluent. Fractions containing the product were pooled and evaporated in vacuo to give the desired product (28 mg, 43%) as a colorless solid. ^{1}H NMR (acetonitrile-d_3): δ 0.69 (s, 3H), 3.46−4.00 (overlapping m, 7H), 5.83 (br s, 2H), 6.06 (s, 1H), 7.45 (s, 1H), 8.05 (s, 1H). HRMS: calcd for $C_{12}H_{15}BrN_4O_4 + H^+$ 359.0355, found: 359.0350.

4-Chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-*β*-D-ribofuranosyl]-5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (29). To an ice-cold solution of 3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-1-*O*-methyl-α-D-ribofuranose (**5**) (1.06 g, 2.1 mmol) in dichloromethane (30 mL) was added HBr (5.7 M in acetic acid; 2.2 mL) dropwise. The reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 2 h, concentrated in vacuo, and coevaporated with toluene (2 × 15 mL). The resulting oil was dissolved in acetonitrile (10 mL) and was added dropwise into a solution of the sodium salt of 4-chloro-5-methyl-1*H*-pyrrolo[2,3-*d*]pyrimidine in acetonitrile (generated in situ from 4-chloro-5-methyl-1*H*-pyrrolo[2,3-*d*]-pyrimidine) (0.62 g, 3.7 mmol) in anhydrous acetonitrile (70 mL), and NaH (60% in mineral oil, 148 mg, 3.7 mmol), after 2 h of vigorous stirring at room temperature). The combined mixture was stirred at room temperature for 1 day and then evaporated to dryness. The residue was suspended in water (100 mL) and extracted with ethyl acetate (2 × 150 mL). The combined extracts were washed with brine (50 mL), dried over Na_2SO_4, filtered, and evaporated. The crude product was purified on silica gel using hexane/ethyl acetate (9:1, 5:1, 3:1) gradient as the eluent to give the desired product (0.87 g, 66%) as a colorless foam. ^{1}H NMR (DMSO-d_6): δ 0.94 (s, 3H,), 2.31 (s, 3H), 3.80 (dd, J = 2.1 Hz, 11.2 Hz, 1H), 4.01 (dd, J = 1.8 Hz, 11.2 Hz, 1H), 4.26 (m, 2H), 4.62, 4.70 (2d, J = 12.5 Hz, 2H), 4.72, 4.81 (2d, J = 12.1 Hz, 2H), 5.30 (s, 1H), 6.39 (s, 1H), 7.26−7.45 (m, 7H), 8.56 (s, 1H). HRMS: calcd for $C_{27}H_{24}Cl_5N_3O_4 + H^+$ 630.0288, found 630.0276.

4-Chloro-5-methyl-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (30). To a solution of 4-chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-*β*-D-ribofuranosyl]-5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (**29**) (0.87 g, 0.9 mmol) in dichloromethane (30 mL) at −78 °C was added boron trichloride (1 M in dichloromethane, 9.0 mL, 9.0 mmol) dropwise. The mixture was stirred at −78 °C for 2.5 h and then at −30 to −20 °C for 3 h. The reaction was quenched by addition of methanol/dichloromethane (1:1) (9 mL), and the resulting mixture was stirred at −15 °C for 30 min, and then neutralized with aqueous ammonia at 0 °C and stirred at room temperature for 15 min. The solid was filtered and washed

with dichloromethane/methanol (1:1, 50 mL). The combined filtrate was evaporated in vacuo, and the residue was purified on silica gel using dichloromethane and dichloromethane/methanol (40:1 through 30:1) as the eluent to furnish the desired compound (0.22 g, 78%) as colorless foam. ^{1}H NMR (DMSO-d_6): δ 0.62 (s, 3H), 2.36 (s, 3H), 3.58−3.94 (m, 4H), 5.04−5.14 (m, 3H), 6.18 (s, 1H, H-1′), 7.77 (s, 1H), 8.54 (s, 1H). HRMS: calcd for $C_{13}H_{16}ClN_3O_4 + H^+$ 314.0908, found 314.0905.

4-Amino-5-methyl-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (31). To 4-chloro-5-methyl-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**30**) (0.2 g, 0.64 mmol) was added methanolic ammonia (saturated at 0 °C) (40 mL). The mixture was heated in a stainless steel autoclave at 100 °C for 14 h and then cooled and evaporated in vacuo. The crude mixture was purified on silica gel with dichloromethane/methanol (30:1, 20:1) gradient as the eluent to give the title compound (0.13 g, 69%) as a colorless solid. ^{1}H NMR (DMSO-d_6): δ 0.60 (s, 3H), 2.26 (s, 3H), 3.52−3.61 (m, 1H), 3.70−3.88 (m, 3H), 5.00 (s, 1H), 4.91−4.99 (m, 3H), 6.04 (s, 1H), 6.48 (br s, 2H), 7.12 (s, 1H), 7.94 (s, 1H). HRMS: calcd for $C_{13}H_{18}N_4O_4 + H^+$ 295.1406, found 295.1407.

4-Amino-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo-[2,3-*d*]pyrimidine-5-carboxylic acid (34). 4-Amino-5-methyl-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**34**) (0.035 g, 0.11 mmol) was dissolved in a mixture of aqueous ammonia (4 mL, 30 wt %) and saturated methanolic ammonia (2 mL), and a solution of H_2O_2 in water (2 mL, 35 wt %) was added. The reaction mixture was stirred at room temperature for 18 h. The solvent was removed under reduced pressure, and the residue was purified by HPLC on a reverse-phase column (Altech Altima C-18, 10 mm × 299 mm, A = water, B = acetonitrile, 10 to 60% B in 50 min, flow 2 mL/min) to yield the title compound (0.015 g, 41%) as a white solid. ^{1}H NMR (methanol-d_4): δ 0.85 (s, 3H), 3.61 (m, 1H), 3.82 (m, 1H) 3.99−4.86 (m, 2H), 6.26 (s, 1H), 8.10 (s, 2H) 8.22 (s, 1H). HRMS (FAB): calcd for $C_{13}H_{16}N_4O_6 + H^+$ 325.1148, found 325.1143.

2,4-Diamino-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (35). 2-Amino-4-chloro-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**19**) (21 mg, 0.067 mmol) and concentrated aqueous ammonia (10 mL) were heated in a stainless steel autoclave overnight at 95 °C and then cooled and evaporated in vacuo. The crude mixture was purified on a silica gel using dichloromethane/methanol (10:1 through 5:1) as the eluent to give the title compound (11 mg, 56%) as a colorless solid. ^{1}H NMR (DMSO-d_6): δ 0.68 (s, 3H), 3.48−3.58 (m, 1H), 3.68−3.73 (m, 2H), 3.84 (m, 1H), 4.72 (s, 1H), 4.97−5.03 (m, 2H), 5.45 (s br, 2H), 6.00 (s, 1H), 6.28 (d, J = 3.7 Hz, 1H), 6.44 (br s, 2H), 6.92 (d, J = 3.7 Hz, 1H). HRMS: calcd for $C_{12}H_{17}N_5O_4 + H^+$ 296.1359, found 296.1358.

4-Amino-2-fluoro-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (36). To a solution of HF/pyridine (70%, 2 mL) and pyridine (0.5 mL) at −25 °C was added 2,4-diamino-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**35**) (60 mg, 0.20 mmol) in pyridine (0.5 mL) followed by *tert*-butyl nitrite (0.036 mL). Stirring was continued for 5 min at −25 °C, and the solution was then poured into ice water (5 mL), neutralized with 2 N aqueous NaOH, and evaporated in vacuo. The residue was purified on silica gel using dichloromethane/methanol (20:1 to 10:1) as the eluent to give the desired compound (18 mg, 30%) as a colorless powder. ^{1}H NMR (acetonitrile-d_3): δ 0.80 (s, 3H), 3.46 (t, 1H), 3.52 (d, 1H), 3.58 (s, 1H), 3.72−3.82 (m, 1H), 3.85−4.01 (m, 2H), 4.13 (dd, 1H), 6.04 (s, 1H), 6.09 (br s, 2H), 6.55 (d, 1H), 7.37 (d, H). HRMS: calcd for $C_{12}H_{15}FN_4O_4 + H^+$ 299.1156, found 299.1157.

2-Amino-5-chloro-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (37). To a precooled solution (0 °C) of 2-amino-4-chloro-7-(2-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**19**) (31 mg, 0.10 mmol) in dimethylformamide (0.5 mL) was added *N*-chlorosuccinimide (14.3 g, 0.11 mmol) in dimethylformamide (0.5 mL) dropwise. The solution was stirred at room temperature for 2 h, and the reaction was quenched by addition of methanol (5 mL) and evaporated in vacuo. The crude product was purified on silica gel using methanol/dichloromethane (1:9) as the eluent. Fractions containing the product were pooled and evaporated in vacuo. This product was dissolved in 2 N aqueous NaOH (2 mL) and heated at reflux for 2 h. The suspension was cooled and neutralized with 2 N aqueous HCl. The suspension was absorbed on silica gel and was purified on a silica gel column using methanol/dichloromethane (1:9 through 1:5) as the eluent. Fractions containing the product were pooled and evaporated in vacuo to give the desired compound (14 mg, 42%) as a colorless solid. ^{1}H NMR (methanol-d_4): δ 0.85 (s, 3H), 3.77 (m, 1H), 3.90−4.07 (overlapping m, 2H), 6.05 (s, 1H), 7.13 (s, 1H). HRMS calcd for $C_{12}H_{15}ClN_4O_5 + H^+$ 331.0809, found 331.0815.

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Supporting Information Available: Tables of experimental details for compound **9**, atomic parameters, and interatomic distances and angles. This material is available free of charge via the Internet at http://pubs.acs.org.

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