# Structure−Activity Relationship of Purine Ribonucleosides for Inhibition of Hepatitis C Virus RNA-Dependent RNA Polymerase

Anne B. Eldrup,*,† Charles R. Allerson,† C. Frank Bennett,† Sanjib Bera,† Balkrishen Bhat,† Neelima Bhat,† Michele R. Bosserman,‡ Jennifer Brooks,† Christine Burlein,‡ Steven S. Carroll,‡ P. Dan Cook,† Krista L. Getty,‡ Malcolm MacCoss,§ Daniel R. McMasters,§ David B. Olsen,‡ Thazha P. Prakash,† Marija Prhavc,† Quanlai Song,† Joanne E. Tomassini,‡ and Jie Xia†

*Department of Medicinal Chemistry, Isis Pharmaceuticals, 2280 Faraday Avenue, Carlsbad, California 92008, Department of Biological Chemistry, Merck Research Laboratories, West Point, Pennsylvania 19486, and Department of Medicinal Chemistry, Merck Research Laboratories, Rahway, New Jersey 07065*

*Received September 2, 2003*

As part of a continued effort to identify inhibitors of hepatitis C viral (HCV) replication, we report here the synthesis and evaluation of a series of nucleoside analogues and their corresponding triphosphates. Nucleosides were evaluated for their ability to inhibit HCV RNA replication in a cell-based, subgenomic replicon system, while nucleoside triphosphates were evaluated for their ability to inhibit in vitro RNA synthesis mediated by the HCV RNA-dependent RNA polymerase, NS5B. 2′-*C*-Methyladenosine and 2′-*C*-methylguanosine were identified as potent inhibitors of HCV RNA replication, and the corresponding triphosphates were found to be potent inhibitors of HCV NS5B-mediated RNA synthesis. The data generated in the cell-based assay demonstrated a fairly stringent structure−activity relationship around the active nucleosides. Increase in steric bulk beyond methyl on C2, change in the stereo- or regiochemistry of the methyl substituent, or change of identity of the heterobase beyond that of the endogenous adenine or guanine was found to lead to loss of inhibitory activity. The results highlight the importance of the ribo configuration 2′- and 3′-hydroxy pharmacophores for inhibition of HCV RNA replication in the cell-based assay and demonstrate that inclusion of the 2′-*C*-methylribonucleoside pharmacophore leads to increased resistance to adenosine deaminase and purine nucleoside phosphorylase mediated metabolism.

## Introduction

The hepatitis C virus (HCV) is the pathogen associated with the majority of sporadic and transfusion-related non-A and non-B hepatitis infections. While often asymptomatic, HCV infection can progress to chronic hepatitis, leading to liver cirrhosis and sometimes hepatocellular carcinoma. Current estimates suggest that 170 million people worldwide suffer from HCV infection. At present, treatment options comprise immunotherapy using recombinant interferon-α in combination with ribavirin. The clinical benefit of this treatment is limited, and a vaccine has not yet been developed. Consequently, HCV infection represents a significant health problem in need of more effective therapies.

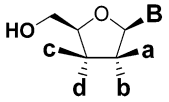
The hepatitis C virus genome consists of a single open reading frame encoding a >3000 amino acid protein that is processed into multiple viral proteins including the NS2/3 autoprotease, the NS3 serine protease and NTPase/helicase, and NS5B, the RNA-dependent RNA polymerase (RdRp).[1−3] Significant effort has been directed toward the identification of specific inhibitors of the HCV protease NS3,[4−9] revealing several, very potent inhibitors. The clinical evaluation of these inhibitors is ongoing and hence has yet to result in marketed therapies. The RNA-dependent RNA polymerase, encoded at the 3′-terminal portion of the HCV genome, is essential to viral replication and thus represents a valid target for therapeutic intervention by design of specific inhibitors.

Nucleoside inhibitors (NIs) of virally encoded polymerases have been validated for other viral targets, such as HIV. While a number of non-nucleoside inhibitors (NNIs) of NS5B-mediated HCV RNA replication have been reported,[10−13] only a few NIs have been identified.[14−16] Thus far, most NS5B NIs described have involved some alteration of the 2′- or 3′-position. Virtually all NIs of virally encoded polymerases described thus far elicit their effect through "chain termination". In order for a nucleoside triphosphate to effect chain termination, the nucleoside triphosphate would need to initially serve as a substrate for HCV NS5B, leading to incorporation of the nucleoside monophosphate and subsequent translocation within the active site in preparation for extension of the growing RNA chain. Second, the nucleoside triphosphate would need to encompass one or more structural elements that would effect "chain termination", that is, significantly impede the further elongation of the RNA chain. Structural elements that influence the 3′-position sterically or electronically would be expected to affect elongation efficiency because the 3′-hydroxyl group participates directly in phosphodiester formation as part of the elongation process. The requirement for HCV NS5B to be able to distinguish ribonucleoside triphosphates from

* To whom correspondence should be addressed. Phone: 760 603 3852. Fax: 760 603 4654. E-mail: aeldrup@isisph.com.
† Isis Pharmaceuticals.
‡ Merck Research Laboratories, PA.
§ Merck Research Laboratories, NJ.

10.1021/jm030424e CCC: $27.50 
Published on Web 03/26/2004

**Table 1.** Inhibitory Potency of 2′- and 3′-Modified Nucleoside Triphosphates on NS5B-Catalyzed RNA Synthesis (NS5B $IC_{50}$) and Inhibitory Potency of 2′- and 3′-Modified Nucleosides on HCV RNA Replication in a Cell-Based, Subgenomic Replicon Assay (Replicon $EC_{50}$)




| | a | b | c | d | B | NS5B $IC_{50}$, $\mu$M | replicon[a] $EC_{50}$, $\mu$M |
|---|---|---|---|---|---|---|---|
| **1** | H | OH | H | H | adenine | 22 | 46 |
| **2** | H | OH | H | H | guanine | 0.6 | 50 |
| **3** | H | OH | H | F | adenine | 28 | 1.2[b] |
| **4** | H | OH | H | F | guanine | 1.8 | >50 |
| **5** | H | $OCH_3$ | H | OH | adenine | 47 | >50 |
| **6** | H | $OCH_3$ | H | OH | guanine | 1.6 | >50 |

[a] Replicon data represent the value at 24 h. [b] Although toxicity was not observed at up to 50 $\mu$M in replicon cells at 24 h, significant cytotoxicity was observed following longer-term culture in several cell lines.

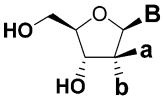
2′-deoxynucleoside triphosphates would make it likely that the polymerase be highly refined in its ability to recognize the 2′-position of nucleoside triphosphates.

As part of a continued effort to identify competitive inhibitors of HCV NS5B, we report here the synthesis and evaluation of a series of nucleoside analogues and their corresponding nucleoside triphosphates. On the basis of the considerations outlined above, our initial SAR was focused on the evaluation of modifications at the 2′ and 3′ positions of the nucleoside (triphosphate). Since some polymerases are known to promote high error rates as a way to provide diversity to the viral genome,[17] we considered it possible that HCV NS5B would be somewhat amenable to changes in the purine heterocycle. Hence, in addition to the SAR at nucleoside 2′ and 3′ positions, a limited SAR was performed to evaluate the effect of modulation of hydrogen-bonding capacity. The ability of the nucleosides to inhibit HCV RNA replication was evaluated in a cell-based, subgenomic HCV replicon assay as previously described.[14] Nucleoside triphosphates were evaluated for their ability to inhibit HCV NS5B-mediated RNA elongation as previously described.[14] For the adenosine derivative that proved active in the cell-based assay, the ability to resist adenosine deaminase (ADA) mediated conversion to the corresponding inosine derivative was assessed. Similarly, the two most active purine derivatives were evaluated for their resistance to purine nucleoside phosphorylase (PNP) mediated phosphorolysis.

## Chemistry

For the initial SAR, a series of nucleoside triphosphates modified at the 2′ or 3′ positions (Tables 1 and 2) were synthesized from the corresponding nucleosides and purified according to the general procedure for triphosphate synthesis, purification, and QC described in the Experimental Section. The following nucleosides were synthesized according to available literature: 3′-deoxy-3′-fluoroadenosine (**3**),[18] 3′-deoxy-3′-fluoroguanosine (**4**),[18] 2′-deoxy-2′-fluoroadenosine (**7**),[19] and 2′-deoxy-2′-fluoroguanosine (**8**)[19] (Table 1). The nucleoside 2′-*C*-methyladenosine (**11**, Table 2) was synthesized using a route similar to that described in ref 20. For the corresponding guanosine derivative (**12**, Table 2), a

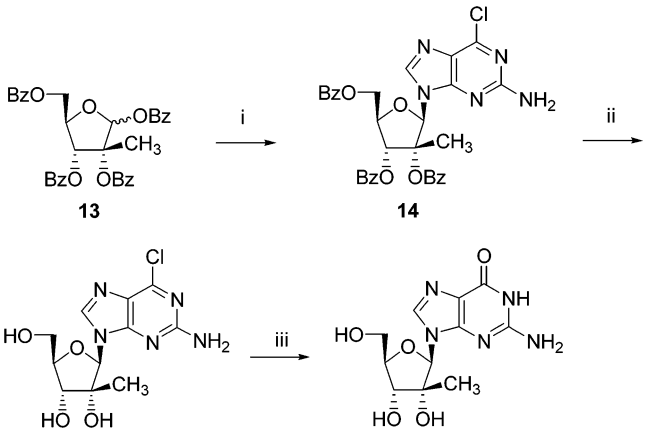
**Table 2.** Inhibitory Potency of 2′-Modified Nucleoside Triphosphates on NS5B-Catalyzed RNA Synthesis (NS5B $IC_{50}$) and Inhibitory Potency of 2′-Modified Nucleosides on HCV RNA Replication in a Cell-Based, Subgenomic Replicon Assay (Replicon $EC_{50}$)




| | a | b | B | NS5B $IC_{50}$, $\mu$M | replicon[a] $EC_{50}$, $\mu$M |
|---|---|---|---|---|---|
| **5** | H | $OCH_3$ | adenine | 47 | >50 |
| **6** | H | $OCH_3$ | guanine | 1.6 | >50 |
| **7** | H | F | adenine | >50 | >50 |
| **8** | H | F | guanine | substrate | >50 |
| **9** | OH | H | adenine | 50 | >50 |
| **10** | OH | H | guanine | 20 | >50 |
| **11** | $CH_3$ | OH | adenine | 1.9 | 0.3 |
| **12** | $CH_3$ | OH | guanine | 0.13 | 3.5 |

[a] Replicon data represent the value at 24 h.

**Scheme 1.** Synthetic Route to 2′-*C*-methylguanosine, **12**[a]

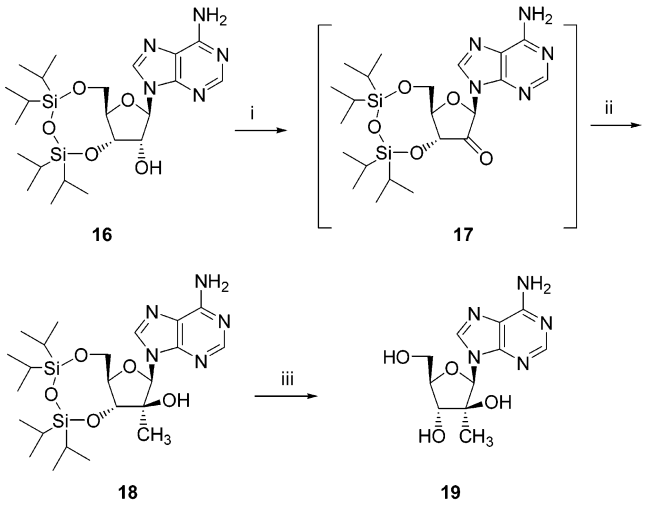



[a] Reagents and conditions: (i) 2-amino-6-chloropurine, DBU and trimethylsilyl triflate in actonitrile, 4 h at 65 °C; (ii) methanolic ammonia, 7 h at room temperature; (iii) 2-mercaptoethanol and sodium methoxide in methanol, 7 h at reflux.

similar strategy was applied (Scheme 1). Briefly, glycosylation of 2-*C*-methyl-1,2,3,5-tetra-*O*-benzoyl-D-ribose[21] (**13**) under Vorbrüggen conditions yielded the benzoyl-protected 2-amino-6-chloropurine derivative (**14**) under stereocontrol induced by transient 1,2-acyloxonium ion formation. Deprotection of this derivative gave the ribonucleoside (**15**), which was readily converted to 2′-*C*-methylguanosine (**12**) by substitution of the 6-chloro substituent with hydroxide.

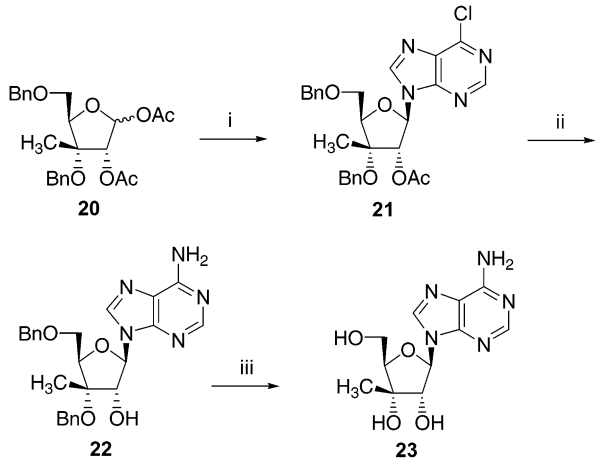
Addition of organometallics to 2-keto ribofuranose intermediates is known to proceed stereoselectively with addition of the alkyl group to the $\beta$ face, leading to formation of the ribo derivatives, as illustrated by the synthesis of **12** (Scheme 1).[21] Consequently, to explore the effect of inversion of stereochemistry at C2′, we utilized "the nucleoside route" proceeding via the previously reported and unstable 2-keto derivative.[22] Thus, adenosine was 3′,5′-*O*-protected with the bifunctional reagent 1,3-dichloro-1,1,3,3,-teraisopropyldisiloxane (TIPDS dichloride) to give **16**[23] (Scheme 2), which was subsequently oxidized under Jones conditions to give the 2-keto intermediate (**17**). This derivative was reacted with methylmagnesium bromide to afford the

**Scheme 2.** Synthetic Route to 2′-*C*-methylarabinoadenosine, **19**[a]




[a] Reagents and conditions: (i) $CrO_3$, pyridine, acetic anhydride in dichloromethane, 30 min at 0 °C; (ii) methylmagnesium bromide in diethyl ether/tetrahydrofuran, 4 h at −30 to −50 °C; (iii) hydrogen fluoride/triethylamine in dichloromethane/acetonitrile, 3 h at room temperature.

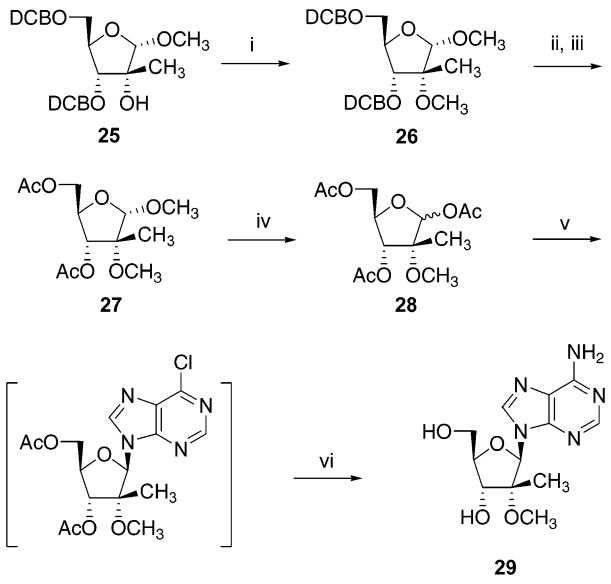
**Scheme 3.** Synthetic Route to 3′-*C*-methyladenosine, **23**[a]




[a] Reagents and conditions: (i) 6-chlorpurine, DBU and trimethylsilyl triflate in acetonitrile, 3 h at 60 °C; (ii) liquid ammonia in dioxane, 14 h at 80 °C; (iii) ammonium formate and Pd/C in methanol, 12 h at reflux.

TIPDS-protected 2′-*C*-methylarabino derivative (**18**) under stereocontrol arising from steric hindrance of the $\beta$ face of the sugar by the purine heterocycle. Subsequent deprotection with hydrogen fluoride gave the desired 2′-*C*-methylarabinoadenosine (**19**). To assess the effect of change in regiochemistry for the methyl substituent, 3′-*C*-methyladenosine (**23**) and 3′-*C*-methylxyloadenosine (**24**) were synthesized. 3′-*C*-Methyladenosine (**23**) was synthesized from **20**[24] according to the procedure outlined in Scheme 3. Stereospecific coupling of **20** with 6-chloropurine under Vorbrüggen conditions yielded the fully protected derivative **21**. Subsequent ammonolysis with liquid ammonia at elevated temperature gave **22** and was followed by deprotection under hydrogen-transfer conditions to give the desired 3′-*C*-methyladenosine (**23**). 3′-*C*-Methylxyloadenosine (**24**) was synthesized by a method similar to that described in ref 25.

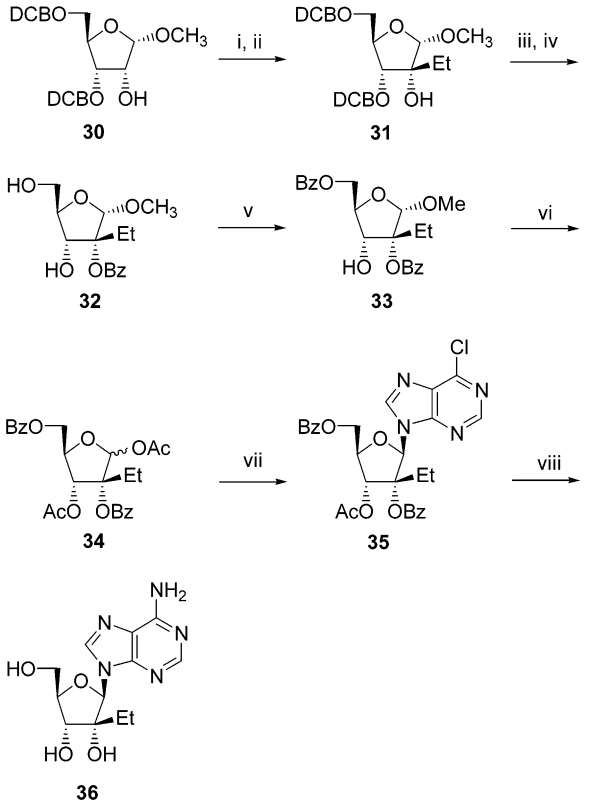
**Scheme 4.** Synthetic Route to 2′-*C*-methyl-2′-*O*-methyladenosine, **29**[a]




[a] Reagents and conditions: (i) KOH, 18-crown-6 and MeI in tetrahydrofuran, 3 h at room temperature; (ii) Pd/C, hydrogen, dichloromethane/methanol/acetic acid, 24 h at room temperature; (iii) acetic anhydride-pyridine (1:1), DMAP, 16 h at room temperature; (iv) acetic anhydride, concentrated $H_2SO_4$, 1 h at 0 °C; (v) 6-chloropurine, trimethylsilyl triflate, DBU, acetonitrile, 5 h at 65 °C; (vi) methanolic ammonia, 16 h at room temperature.

The effect of methylation of the 2′-hydroxyl group was investigated by the synthesis of 2′-*C*-methyl-2′-*O*-methyladenosine (**29**) according to the route depicted in Scheme 4. Starting with the previously reported 3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-1-*O*-methyl-α-D-ribofuranose (**25**),[26,27] methylation was performed under phase-transfer conditions (KOH, 18-crown-6 and methyl iodide) to give **26**. The subsequent change of protection groups from 2,6-dichlorobenzyl to acetyl proceeded via catalytic hydrogenation and acetylation under standard conditions to give **27**. Insertion of the acetate leaving group at C1 was performed under acidic conditions to yield the key intermediate **28**. Coupling under Vorbrüggen conditions was performed to yield a mixture of $\alpha/\beta$ diastereoisomers. Separation of the diastereoisomeric mixture was accomplished after deprotection and substitution of the 6-chloro functionality, using methanolic ammonia at elevated temperature, to give the desired $\beta$ anomer of 2′-*C*-methyl-2′-*O*-methyladenosine (**29**) as well as its α anomer. In an effort to further explore the structural effects at the furanose C2′, 2′-*C*-ethyladenosine was also synthesized (**36**, Scheme 5). An initial attempt to employ the strategy used in the synthesis of the purine 2′-*C*-methylribonucleosides **11** and **12** failed, possibly because of failure to form the prerequisite $EtTiCl_3$ species. As an alternative to using this relatively nonbasic, chemospecific titanium reagent, we opted to instead use the ribose precursor with the less-sensitive dichlorobenzyl protecting group, which allowed the use of the more reactive EtMgBr. Synthesis of **31** was subsequently achieved via the stereospecific addition of the ethyl substituent to the $\beta$ face of the previously described 3,5-bis-*O*-(2,4-dichlorophenylmethyl)-1-*O*-methyl-α-D-*erythro*-pentofuranose-2-ulose (**30**)[26,27] using EtMgBr in diethyl ether. Benzoylation of

**Scheme 5.** Synthetic Route to 2′-*C*-ethyladenosine, **36**[a]

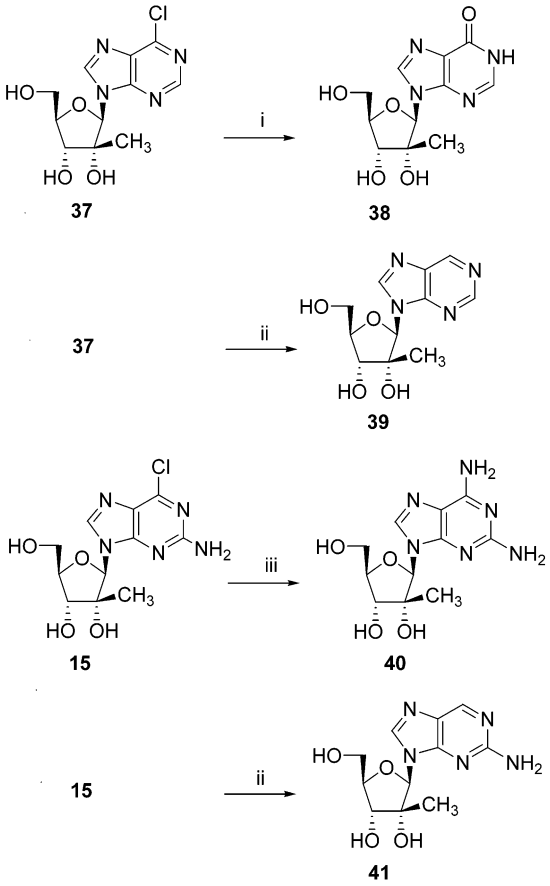



[a] Reagents and conditions: (i) Dess−Martin periodinane, dichloromethane, 48 h at room temperature; (ii) EtMgBr, diethyl ether, 4 h at −78 to −15 °C; (iii) benzoyl chloride, DMAP, triethylamine, dichloromethane, 48 h at 50 °C; (iv) Pd/C, hydrogen, methanol/acetic acid (5:2); (v) benzoyl chloride, DMAP, triethylamine, dichloromethane, 3 h at room temperature; (vi) acetic acid, acetic anhydride, $H_2SO_4$, overnight at room temperature; (vii) 6-chloropurine, trimethylsilyl triflate, DBU, acetonitrile, 1.5 h at 50 °C; (viii) ammonia/dioxane (5:1), overnight at 50 °C.

the sterically hindered 2-hydroxyl group was performed under forcing conditions, followed by catalytic hydrogenation and subsequent esterification of the 5-hydroxyl functionality to give **33**. Hydrolysis of the ketal was succeeded by acetylation to establish the acetate leaving group at C1 (necessary for stereospecific coupling of the purine base) and simultaneous esterification of the 2-hydroxyl group to give the derivative **34**. This derivative was coupled under Vorbrüggen conditions to generate the protected derivative **35**. Ammonolysis and deprotection were achieved in a single step using liquid ammonia at elevated temperature to give the desired 2′-*C*-ethyladenosine (**36**).

The purine ribonucleoside derivatives **38**−**41** (Scheme 6) were prepared in order to evaluate the effect of changes in the hydrogen bond acceptor and donor sites of the purine heterobase. 2′-*C*-Methylinosine (**38**) was readily obtained from hydrolytic substitution on the 6-chloropurine derivative **37**,[20] while the purine derivative **39** was available through catalytic hydrogenation of the same 6-chloropurine precursor. The remaining members of this series were prepared from the 2-amino-6-chloropurine-2′-*C*-methyl ribonucleoside **15** either through ammonolysis to yield the 2,6-diaminopurine derivative (**40**) or by catalytic hydrogenation to yield the 2-aminopurine derivative (**41**).

**Scheme 6.** Synthetic Route to Heterobase Modified 2′-*C*-methylribonucleosides, **38**−**41**[a]




[a] Reagents and conditions: (i) 1 N aqueous sodium hydroxide, 2 h at reflux; (ii) Pd/C and triethylamine in methanol under hydrogen, overnight at room temperature; (iii) ammonium hydroxide, overnight at 80 °C.

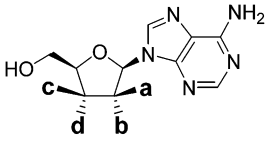
## Results

A series of nucleoside triphosphates, modified in the furanosyl 2′- or 3′-positions, were screened against the purified HCV NS5B in order to assess their ability to inhibit HCV NS5B-mediated RNA synthesis (Table 1). The corresponding nucleosides were screened in a cell-based, subgenomic replicon assay for the ability to inhibit HCV RNA replication; in this assay, activity would be dependent on both cellular uptake and the cell's ability to metabolize the nucleoside to its triphosphate (Table 1). While none of the screened nucleosides demonstrated significant activity in the cell-based assay ($EC_{50}$ values ranging from 46 to >50 $\mu$M) [apart from 3′-deoxy-3′-fluoroadenosine ($EC_{50} = 1.2$ $\mu$M) which was quite cytotoxic in long-term cell culture], several nucleoside triphosphates in the guanine series proved to be low- or submicromolar inhibitors of HCV NS5B-mediated RNA synthesis. As might be expected, a number of nucleoside triphosphates lacking the 3′-hydroxyl moiety were potent inhibitors of HCV NS5B-mediated RNA synthesis as illustrated by the derivatives 3′-deoxyguanosine (Table 1, **2**, $IC_{50} = 0.6$ $\mu$M) and 3′-deoxy-3′-fluoroguanosine (Table 1, **4**, $IC_{50} = 1.8$ $\mu$M). The observed inhibition of RNA synthesis most likely occurs via incorporation of the nucleoside monophosphates and subsequent inability to support further extension of the growing RNA chain (chain termination).

Somewhat surprisingly, alkylation of the 2′-hydroxyl group in guanosine triphosphate to the corresponding 2′-*O*-methylguanosine triphosphate (Table 1, **6**, $IC_{50}$ = 1.6 $\mu$M) also resulted in a potent inhibitor of RNA synthesis. In contrast, 2′-*O*-methyladenosine triphosphate (Table 1, **5**, $IC_{50}$ = 47 $\mu$M) failed to inhibit HCV NS5B-mediated RNA synthesis.

**Modifications of the Ribose 2-Position.** On the basis of the finding that modification of the 2′-position of ribonucleoside triphosphates could produce potent chain terminators, we elected to further explore the structural space around this position. While 2′-*O*-methylguanosine triphosphate (Table 2, **6**) was found to be a potent inhibitor of HCV NS5B-mediated RNA synthesis, the corresponding 2′-deoxy-2′-fluoroguanosine triphosphate derivative (Table 2, **8**, substrate) supported RNA elongation following its incorporation into the RNA chain. As before, the corresponding adenosine derivative 2′-deoxy-2′-fluoroadenosine triphosphate (Table 2, **7**, $IC_{50}$ > 50 $\mu$M) derivative was found to be inactive. Since both the 2′-*O*-methyl and 2′-deoxy-2′-fluoro modifications eliminate the 2′-hydroxy hydrogen bond donor, it might be speculated that the ability of **6** to effect chain termination is caused by steric interference between the 2′-*O*-methyl substituent and either the polymerase or the incoming nucleoside triphosphate. The 2′-fluoro modification might not cause sufficient steric hindrance to trigger inhibition of RNA synthesis by such a mechanism. Alternatively, the disparity in activity might be the result of differences in conformational preferences of the two modified furanoses.[28] To probe the effect of substituents on the $\beta$ face of the nucleoside, we tested nucleosides and nucleoside triphosphates in which the 2′-hydroxyl group was inverted from the endogenous ribo configuration to the arabino orientation (Table 2, **9** and **10**). With both adenosine and guanosine derivatives, activity was absent in the cell-based assay and, for the triphosphates, was significantly reduced against HCV NS5B. Only the arabinoguanosine triphosphate displayed a weak inhibitory effect on HCV NS5B-mediated RNA synthesis (Table 2, **10**, $IC_{50}$ = 20 $\mu$M). Thus, it appeared that while increased steric bulk around the 2′-position could create nonobligate chain terminators, exclusion of the hydrogen bond donor/acceptor in the 2′-ribo position generally led to significantly reduced, or in some cases complete abrogation of inhibitory activity. This, in combination with the observation that potency of the 3′-deoxy modified nucleosides in the enzymatic assay did not translate into inhibition in the cell-based assay, led us to examine the 2′-*C*-methyl derivatives **11** and **12** (Table 2). In both of these derivatives, the ribo configuration of the 2′- and 3′-hydroxyl groups were maintained as recognition elements, while the $\beta$ face of the nucleoside was sterically compromised by addition of a 2′-*C*-methyl substituent. This class of compounds demonstrated potent activity in the cell-based assay, as well as against HCV NS5B. For 2′-*C*-methyladenosine (Table 2, **11**), inhibition of the enzyme by the triphosphate was moderate ($IC_{50}$ = 1.9 $\mu$M), while the activity observed in the cell-based assay was comparably better ($EC_{50}$ = 0.3 $\mu$M). The opposite relationship was observed for the corresponding 2′-*C*-methylguanosine derivative ($IC_{50}$ = 0.13 $\mu$M, $EC_{50}$ = 3.5 $\mu$M) (Table 2, **12**).

**Table 3.** Inhibitory Potency of Modified Nucleoside Triphosphates, Modified by Inclusion of Alkyl Substituents To Give Alkyl-Derivatized Ribo, Ara, and Xylo Derivatives, on NS5B-Catalyzed RNA Synthesis (NS5B $IC_{50}$) and Inhibitory Potency of Nucleosides, Modified by Inclusion of Alkyl Substituents To Give Alkyl-Derivatized Ribo, Ara, and Xylo Derivatives, on HCV RNA Replication in a Cell-Based, Subgenomic Replicon Assay (Replicon $EC_{50}$)




| | a | b | c | d | NS5B $IC_{50}$, $\mu$M | replicon[a] $EC_{50}$, $\mu$M |
|---|---|---|---|---|---|---|
| **11** | $CH_3$ | OH | H | OH | 1.9 | 0.3 |
| **19** | OH | $CH_3$ | H | OH | >50 | >50 |
| **23** | H | OH | $CH_3$ | OH | >50 | >50 |
| **24** | H | OH | OH | $CH_3$ | >50 | >50 |
| **29** | $CH_3$ | $OCH_3$ | H | OH | >50 | >50 |
| **36** | Et | OH | H | OH | >50 | >50 |

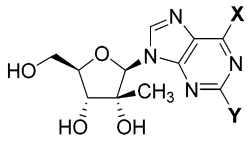
[a] Replicon data represent value at 24 h.

**Methyl Substitutions at the Ribose C2 and C3.** Structurally related analogues of the active adenosine based inhibitor **11** (Table 3) were synthesized in order to assess the effect of changes in stereo- and regiochemistry of the furanose moiety. The importance of the stereochemistry at C2′ was demonstrated through the synthesis and testing of the arabino derivative, which was found to be inactive in the cell-based assay ($EC_{50}$ > 50 $\mu$M) (**19**, Table 3). Likewise, the corresponding triphosphate was found to be inactive against HCV NS5B ($IC_{50}$ > 50 $\mu$M), indicating that the lack of cell-based activity is at least in part due to the enzyme's inability to accept this modification. The importance of regiochemistry in the context of methyl substitution was examined by synthesis of 3′-*C*-methyladenosine (**23**, Table 3) and 3′-*C*-methylxyloadenosine (**24**, Table 3). Both compounds tested inactive in the cell-based assay ($EC_{50}$ > 50 $\mu$M). Similarly, the corresponding triphosphates were synthesized and found to be incapable of inhibiting HCV NS5B-mediated RNA synthesis ($IC_{50}$ > 50 $\mu$M).

**Effect of Methylation of the 2′-Hydroxyl Group and Increased Steric Bulk at C2′.** On the basis of the inactivity of the arabino derivative **19**, the 2′-hydroxyl group of **11** appeared to be an important structural element for activity. This was further investigated by synthesizing and evaluating the inhibitory activity of 2′-*C*-methyl-2′-*O*-methyladenosine (**29**, Table 3). The nucleoside failed to inhibit HCV RNA replication in the cell-based replicon assay ($EC_{50}$ > 50 $\mu$M), as did its α-anomer ($EC_{50}$ > 50 $\mu$M) (data not shown). Furthermore, the corresponding triphosphate displayed no ability to inhibit HCV NS5B-mediated RNA synthesis ($IC_{50}$ > 50 $\mu$M). To more thoroughly examine the effect of increased steric bulk around C2′, 2′-*C*-ethyladenosine (**36**) and the corresponding triphosphate were synthesized. The change in steric bulk was found to be deleterious to both cell-based ($EC_{50}$ > 50 $\mu$M) and enzymatic activities ($IC_{50}$ > 50 $\mu$M).

**Modifications of the Purine Heterobase.** To probe the tolerance for changes in hydrogen-bonding capacity of the purine heterobase, 2′-*C*-methyl modified ribonucleosides were synthesized in which hydrogen bond

**Table 4.** Inhibitory Potency of Heterobase Modified Nucleoside Triphosphates on NS5B-Catalyzed RNA Synthesis (NS5B $IC_{50}$) and Inhibitory Potency of Heterobase Modified Nucleosides on HCV RNA Replication in a Cell-Based, Subgenomic Replicon Assay (Replicon $EC_{50}$)



| | X | Y | NS5B $IC_{50}$, $\mu$M | replicon[a] $EC_{50}$, $\mu$M |
|---|---|---|---|---|
| **11** | $NH_2$ | H | 1.9 | 0.3 |
| **12** | OH | $NH_2$ | 0.13 | 3.5 |
| **38** | OH | H | 4.0 | >50 |
| **39** | H | H | nd | >50 |
| **40** | $NH_2$ | $NH_2$ | 2.6 | 7.0 |
| **41** | H | $NH_2$ | 46 | >50 |

[a] Replicon data represent the value at 24 h.

**Table 5.** Comparison of the Calculated Lowest-Energy Northern- and Southern-Type Conformers for the Structures in Figure 1[a]

| | Northern | | | Southern | | |
|---|---|---|---|---|---|---|
| compd | *E*, kcal $mol^{-1}$ | *P*, deg | $\tau$, deg | *E*,kcal $mol^{-1}$ | *P*, deg | $\tau$, deg |
| **11** | 0 | 15.6 | 38.9 | 2.52 | 155.2 | 35.7 |
| **19** | 2.25 | 14.9 | 37.2 | 0 | 165.0 | 36.1 |
| **23** | 2.91 | 31.6 | 38.0 | 0 | 180.4 | 38.8 |
| **24** | 0 | 26.4 | 36.5 | 1.99 | 184.1 | 37.3 |

[a] *E* is the energy of the conformer relative to the MMFFs ($\epsilon$ = 50) global minimum. *P* is the pseudorotational angle in degrees, and $\tau$ is the puckering amplitude in degrees.

donors and/or acceptors were either omitted or added (**38**−**41**, Table 4). Deletion of the 6-amino group to give the purine ribonucleoside derivative (**39**) led to complete loss of activity in the cell-based assay ($EC_{50}$ > 50 $\mu$M). Similarly, the inosine (**38**) and the 2-aminopurine (**41**) derivatives were also unable to inhibit HCV RNA replication in the cell-based assay ($EC_{50}$ > 50 $\mu$M), while the corresponding triphosphates were either low-micromolar or weak inhibitors of HCV NS5B ($IC_{50}$ = 4.0 and 46 $\mu$M, respectively). Addition of an amino hydrogen bond donor in the purine 2-position to give the 2,6-diaminopurine derivative **40** resulted in moderate activity in the cell-based assay ($EC_{50}$ = 7.0 $\mu$M). The corresponding triphosphate proved to be a low-micromolar inhibitor of RNA synthesis ($IC_{50}$ = 2.6 $\mu$M).

**Calculation of Conformational Preference for 11, 19, 23, and 24.** The relative energies of Northern- and Southern-type conformers were calculated for 2′-*C*-methyladenosine (**11**), 2′-*C*-methylarabinoadenosine (**19**), 3′-*C*-methyladenosine (**23**), and 3′-*C*-methylxyloadenosine (**24**) as described in the Experimental Section (Table 5, Figure 1). Whereas **19** and **23** were calculated to prefer a Southern, C2′-endo conformation by 2−3 kcal/mol, **11** and **24** displayed a similarly strong preference for a Northern conformation according to the computation (see Table 5). For comparison, the Northern and Southern conformers of adenosine were calculated by this method to lie much closer in energy, with a Southern C2′-endo, C3′-exo twist conformer lying ca. 0.5 kcal/mol below the Northern C3′-endo conformer, in general agreement with NMR population analyses (data not shown).[28,29] Thus, the conformational bias appears to be driven by the preference of the methyl group for an equatorial orientation.

**Enzymatic Conversions Mediated by Purine Nucleoside Phosphorylase and Adenosine Deaminase.** Pharmacokinetic data obtained in rat have demonstrated the oral bioavailability of 2′-*C*-methylguanosine (**12**) to be 82%.[16] In contrast, the analogous adenosine derivative (**11**) was found to be undetectable in plasma after per oral dosing, with very rapid clearance (>200 mL $min^{-1}$ $kg^{-1}$) observed after intravenous dosing.[14] The limited bioavailability of 2′-*C*-methyladenosine suggests rapid metabolism of this derivative. To further investigate this possibility, we looked at the ability of 2′-*C*-methyladenosine (**11**) and 2′-*C*-methylguanosine (**12**) to function as substrates for relevant metabolic enzymes. Susceptibility of **11** and **12** to cleavage by purine nucleoside phosphorylase (PNP) to give the nucleoside 1′-phosphate and the purine heterocycle was examined at 1.8 and 18 U/mL at 27 h. For

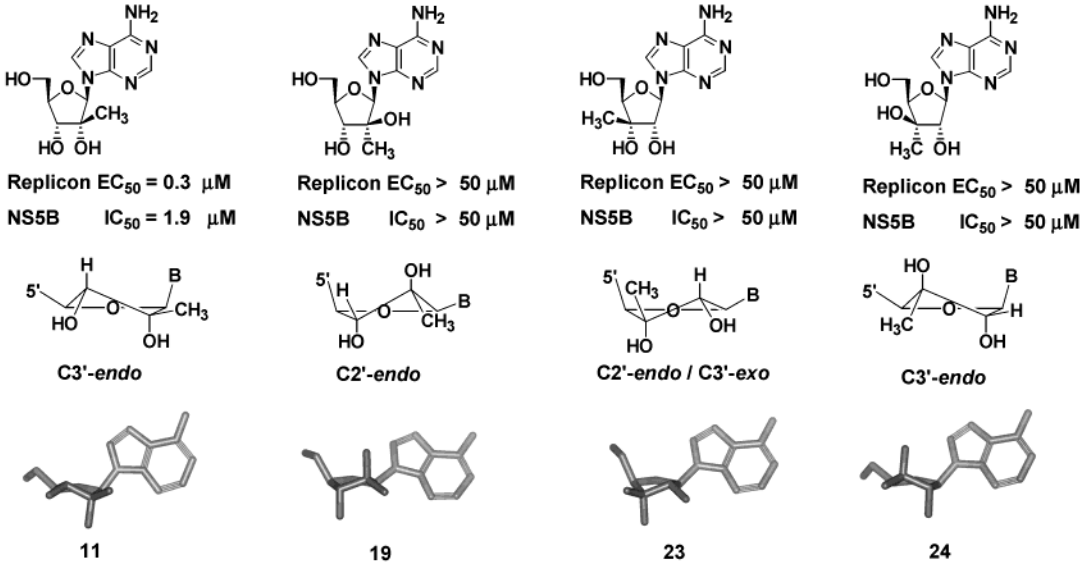



**Figure 1.** Methyl substitution of furanose alters the conformational preference, thereby changing the positioning of the 2′- and 3′-hydroxyl groups. Of the methyl substitutions examined, only **11** retains the ribo configuration 2′- and 3′-hydroxy structure elements as well as the preference for C3′-endo conformation of the furanose.

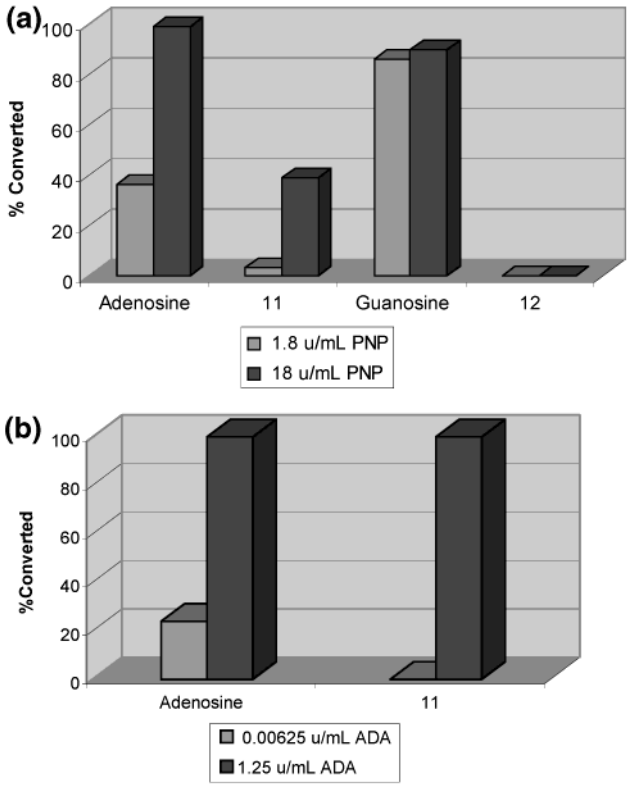



**Figure 2.** (a) Phosphorolysis of the glycosidic bond of nucleosides catalyzed by purine nucleoside phosphorylase (PNP). Nucleosides (200 $\mu$M) were incubated in the presence of enzyme (1.8 or 18 units/mL) for 27 h followed by analysis for product formation using HPLC as described in the experimental procedures. Under the experimental conditions, adenosine, 2′-*C*-methyladenosine (**11**), and guanosine were substrates for PNP-mediated phosphorolysis, while no conversion of 2′-*C*-methylguanosine (**12**) to product was detected. (b) Adenosine deaminase-catalyzed conversion of nucleosides. Adenosine and 2′-*C*-methyladenosine (**11**) (25 $\mu$M) were incubated in the presence of ADA, followed by analysis for product formation using HPLC as described in experimental procedures. Under reaction conditions that include 1.25 units/mL ADA, 2′-*C*-methyladenosine was converted to 2′-*C*-methylinosine.

**12**, no cleavage of the purine was observed (Figure 2a), whereas unmodified guanosine was efficiently cleaved (82% and 87%, respectively, at 1.8 and 18 U/mL PNP). In comparison, **11** was efficiently converted (37%) at the higher enzyme concentration, while unmodified adenosine was almost quantitatively converted (Figure 2a). Similarly, we evaluated the ability of the adenosine derivative **11** to function as a substrate for adenosine deaminase (ADA) mediated conversion to 2′-*C*-methylinosine derivative (Figure 2b). Under conditions in which unmodified adenosine was 25% converted to inosine, 2′-*C*-methyladenosine (**11**) underwent no appreciable conversion. However, at higher enzyme concentrations both adenosine and **11** could be quantitatively converted to their corresponding inosine derivatives.

## Discussion

Of the various modifications to the nucleoside 2′- and 3′-positions examined herein, only the introduction of the 2′-*C*-methyl substituent led to potent inhibition of HCV RNA replication in the cell-based, subgenomic replicon assay. However, several additional 2′- and 3′-modified nucleoside triphosphates demonstrated low or submicromolar activity against the isolated HCV NS5B polymerase. Nucleoside triphosphates based on guanine were generally more active than the corresponding adenine-based derivatives against the NS5B polymerase. The underlying reasons for the functional difference between adenosine and guanosine triphosphate derivatives remain elusive at this time. Deletion of the 3′-hydroxyl group of guanosine to give 3′-deoxy or 3′-deoxy-3′-fluoro derivatives resulted in activity at the enzyme level; however, this activity generally did not translate to activity in the cell-based assay. The inability of the 3′-deoxy modified nucleosides to elicit activity in the cell-based assay is an observation that, in our experience, extends to various classes of nucleosides, both purine- and pyrimidine-derived, in which the 3′-hydroxyl group is excluded (unpublished results). In contrast to the activity generally observed with 3′-deoxyguanosine triphosphates, modification of the 2′-position of nucleoside triphosphates did not generally lead to potent inhibitors of HCV NS5B-mediated synthesis. Of the derivatives examined herein, only 2′-*O*-methylguanosine triphosphate (**6**), 2′-*C*-methyladenosine triphosphate (**11**), and 2′-*C*-methylguanosine triphosphate (**12**) displayed significant inhibitory effect on HCV NS5B-mediated RNA synthesis. For 2′-*O*-methylguanosine, the observed activity of the nucleoside triphosphate did not translate to a matching activity of the nucleoside in the cellular assay. To gain a better understanding of the underlying reason for the observed inactivity of the 2′-*O*-methyl derivative **6** in the cell-based assay, we evaluated cellular uptake and intracellular metabolism for the nucleoside **6**. Uptake and metabolic conversion to the corresponding triphosphate (in hepatoma cells) was found to be inefficient (data not shown). Thus, inefficient cellular uptake and metabolism of 2′-*O*-methylguanosine (**6**) might provide an explanation for the lack of activity of this compound in the cellular assay. The ability of HCV NS5B to discriminate against nucleoside triphosphates modified in the 2′-position would not be surprising in light of the need to avoid the incorporation of 2′-deoxynucleoside monophosphates into the elongated RNA product. However, on the basis of the results presented herein, it appears that HCV NS5B is able to recognize 3′-deoxy modified nucleoside triphosphates and that inactivity of the 3′-deoxynucleosides in the cell-based assay may be the result of upstream selectivity provided by nucleoside transporters and/or nucleoside kinases. Further evaluation of the potent 3′-deoxynucleoside triphosphate NS5B inhibitors, as well as (monophosphate)prodrug approaches to enhance cellular uptake and metabolic profile of the corresponding nucleosides, will be described elsewhere.

While 2′-*C*-methyladenosine triphosphate was found to be a moderately active inhibitor of HCV NS5B-mediated RNA synthesis ($IC_{50}$ = 1.9 $\mu$M), the corresponding nucleoside was found to be comparably more active in the cell-based assay ($EC_{50}$ = 0.3 $\mu$M). The reverse relationship between the two activities was observed for the guanosine derivative ($IC_{50}$ = 0.13 $\mu$M; $EC_{50}$ = 3.5 $\mu$M) (**11** and **12**, Table 2). The observed opposing trends are likely to reflect differences in efficiency of nucleoside uptake and/or intracellular metabolism to the corresponding triphosphate for the

two derivatives. Indeed, uptake and metabolism of the guanosine derivative **12** to the triphosphate in hepatoma cells was found to be lacking,[16] whereas the corresponding adenosine derivative **11** was taken up and processed efficiently under similar conditions.[14] Hence, for these two derivatives, potency in the cellular assay appears to correlate with efficiency of cellular uptake and metabolism to the triphosphate. The fact that cell-based potency tracks with cellular uptake and metabolic conversion to the triphosphate suggests that there may be some room for improving the potency of 2′-*C*-methylguanosine (**12**), perhaps through application of (monophosphate)prodrug approaches. Inefficient uptake of the guanine derivatives would be in line with general observations made during the advancement of this work, indicating a general lower level of uptake for guanosine nucleoside analogues compared to that of other ribonucleoside derivatives (unpublished results).

A change in stereochemistry at C2′, as illustrated by 2′-*C*-methylarabinoadenosine (**19**), was found to result in inactivity in both the cellular and enzymatic assays (**19**, Table 3). The change of stereochemistry at C2′ results in transfer of the 2′-hydroxyl element to the $\beta$ face of the nucleoside, along with a change in the calculated conformational preference to favor C2′-endo. This conformational bias is in contrast to that of the potent inhibitor 2′-*C*-methyladenosine (**11**), which was calculated to prefer a C3′-endo conformation (Table 5, Figure 1). The inability of the triphosphate of **19** to inhibit HCV NS5B-mediated RNA synthesis is also in line with the modest activity of arabinoadenosine (**9**) triphosphate, in which the 2′-hydroxyl group is also positioned on the $\beta$ face of the nucleoside. The regioisomeric 3′-*C*-methyladenosine (**23**, Table 3) and 3′-*C*-methylxyloadenosine (**24**, Table 3) both tested inactive in the cellular assay as well as in the HCV NS5B assay as triphosphates. Because 3′-deoxynucleosides are also inactive in the cellular assay (**1**−**4**, Table 1), the inactivity of derivative **24** in the same cellular assay might be interpreted as confirmation of the requirement for a hydroxyl group with proper stereochemical orientation at C3 for effective inhibition. However, the guanosine derived 3′-deoxynucleoside triphosphates (**2** and **4**, Table 1) are all active inhibitors of HCV RNA synthesis despite the lack of the 3′-hydroxyl functionality. In contrast, the nucleoside triphosphate of **24** was unable to inhibit this enzyme-mediated process. Moreover, 3′-*C*-methyladenosine (**23**), which maintains both hydroxyl groups present in the endogenous adenosine substrate for the polymerase (ATP), was likewise unable to act as an inhibitor (or a substrate) of HCV NS5B-mediated RNA synthesis. The calculated conformational preference of **23** (C2′-endo/C3′-exo) also differs from that of **11** (C3′-endo), which may explain, at least in part, the difference in ability to interact with HCV NS5B (Table 5, Figure 1).

Methylation of the active 2′-*C*-methyladenosine (**12**) to generate 2′-*C*-methyl-2′-*O*-methyl derivative **29** (Table 3) led to a loss in activity in the cell-based assay. The corresponding triphosphate was also unable to inhibit RNA synthesis by HCV NS5B, suggesting that in the context of adenosine derivatives methylation of the 2′-hydroxyl interferes with recognition by HCV NS5B. This interpretation is further supported by the observed inactivity of 2′-*O*-methyladenosine (**5**) in the enzymatic assay. The importance of the 2′-*C*-methyl pharmacophore is accentuated by the complete loss of activity upon addition of a single methylene unit in the closely related ethyl derivative (**36**, Table 3). This derivative was inactive both as a nucleoside in the cellular assay and as a triphosphate in the HCV NS5B assay. Since both the 2′-*C*-methyladenosine (**12**) and the 2′-*C*-ethyladenosine (**36**) derivatives are calculated to exhibit a strong preference for C3′-endo (results not shown), conformational differences cannot explain the observed inactivity of the 2′-*C*-ethyl derivative. Instead, it appears that the enzyme is extremely sensitive to the steric environment surrounding the C2′ position and that the ethyl substituent per se is not tolerated by HCV NS5B. A model derived from the NS5B crystal structure and the $\varphi$6 RdRp initiation complex[16] is consistent with a tight steric environment around C2′ of the nucleoside triphosphate prior to elongation. Effective inhibition of HCV NS5B may require striking a delicate balance between too little (2′-H) and too much (2′-*C*-ethyl) steric bulk, and the size of the methyl substituent may be nearly optimal in this respect.

Some modification of the hydrogen-bonding capacity of the purine heterocycle was found to be tolerated by HCV NS5B without resulting in a complete loss of activity. Simple changes such as the deletion of the 2-amino group of **12** to give the inosine derivative **38** (Table 3), as well as the insertion of an additional hydrogen bond donor in **11** to give the 2,6-diaminopurine derivative **40** (Table 4), were found to create moderate nucleoside triphosphate inhibitors of HCV NS5B-mediated RNA synthesis. Yet, for only one of these derivatives, **40**, did the ability of the nucleoside triphosphate to inhibit RNA synthesis equate with an ability of the nucleoside to inhibit HCV RNA replication in the cell-based assay. However, the observed activity of **40** in the cell-based assay is likely a result of efficient conversion to the corresponding guanosine derivative by adenosine deaminase (data not shown). Thus, the results suggest that HCV NS5B itself has a limited ability to discriminate against changes in the heterobase hydrogen-bonding pattern but that high levels of selection against such modifications can occur upstream from HCV NS5B, in the nucleoside metabolic pathway, possibly by nucleoside receptors and/or nucleoside kinases.

For the two derivatives with demonstrated ability to inhibit HCV RNA replication, **11** and **12**, our data suggest a likely correlation between enzymatic stability and oral bioavailability in rodents.[14,16] 2′-*C*-methylguanosine (**12**) was found to be inert to cleavage of the heterobase by purine nucleoside phosphorylase (PNP), while 2′-*C*-methyladenosine was found to be somewhat susceptible to cleavage by PNP (Figure 2a). Moreover, conversion of 2′-*C*-methyladenosine (**11**) to the corresponding inosine derivative by adenosine deaminase was quite efficient (Figure 2b), indicating that both PNP and ADA mediated conversions may contribute to limiting the oral bioavailability of the adenosine derivative.

In conclusion, 2′-*C*-methyladenosine and 2′-*C*-methylguanosine have been identified as potent inhibitors of HCV RNA replication in a subgenomic replicon assay

harbored in hepatoma cells. While the SAR of the 2′- and 3′-ribonucleoside triphosphate positions appears to be quite stringent, some flexibility exists for modification of the purine heterobase moiety. Our findings indicate that selectivity against heterobase modifications in the cellular assay rely in part on specificity provided by uptake mechanisms and/or kinase activity. Thus, the use of heterobases that have previously demonstrated efficient uptake and conversion to the triphosphate in a ribonucleoside context might prove advantageous in the future design of more potent inhibitors of HCV RNA replication. While modifications of the heterobase in ribonucleosides might be expected to lead to mutagenic events, the 2′-*C*-methyl pharmacophore appears to prevent recognition by human polymerases,[14] which may permit the use of nucleobases otherwise considered unsuitable. Obviously, the success of such an approach would rely on the design of completely metabolically stable 2′-*C*-methylribonucleoside analogues; any ability to function as substrate for ribonucleotide reductase and/or purine nucleoside phosphorylase would open the door for transglycosylation and subsequent incorporation of modified purine nucleosides into the host genome. Additionally, the enzymatic studies presented herein suggest that the oral bioavailability of 2′-*C*-methyladenosine (**11**) may be limited by its conversion to the corresponding inosine derivative assisted by adenosine deaminase (ADA) as well as by PNP-mediated lability of the adenine heterobase. Prodrug approaches may provide one way to minimize conversion by ADA to a level that would permit oral dosing of this compound. More intriguing, however, is the existence of heterobase modifications that are known to be resistant to ADA activity.[30] The use of such heterobase modifications may pave the road for 2′-*C*-methyladenosine derivatives displaying greater potency, improved metabolic profile, and significantly enhanced oral bioavailability relative to 2′-*C*-methyladenosine.

## Experimental Section

**General Methods.** The following chemicals were used as received: 3′-deoxyadenosine (**1**) (Sigma D3394), 3′-deoxyguanosine (**2**) (Sigma D7285), 2′-*O*-methyladenosine (**5**) (Sigma M9886), 2′-*O*-methylguanosine (**6**) (Yamasa Biochemicals 8189), arabinofuranosyladenine (**9**) (Sigma A5762), arabinofuranosylguanine (**10**) (ICN 198924), 2-amino-6-chloropurine (Aldrich, 34,230-0), 6-chloropurine (Aldrich, 51,161-7), 3,5-bis-*O*-(2,4-dichlorophenylmethyl)-1-*O*-methyl-α-D-ribofuranose (Lipomed NUC-55), and tributylammonium pyrophosphate (Sigma P 8533). TLC was performed on silica 60 (Merck 5554 aluminum sheet), and column chromatograpy was performed on silica 60 (230−400 mesh ASTM) (Merck 9385). The 4 Å molecular sieves (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. All nucleosides screened in the call-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, 2−95% 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 triethylammonium acetate, pH 7, B = 10% 100 mM triethylammonium acetate in acetonitrile, 100% B in 25 min, flow 1 mL min$^{-1}$). The purity of the nucleosides screened was generally greater than 98%.

**General Procedure for the Synthesis of Nucleoside Triphosphates.** To the appropriate, unprotected nucleoside (0.05 mmol) was added 4 Å molecular sieves (about 20−30) (Mallinckrodt 4494) and trimethyl phosphate (stored over sieves) (0.5 mL). The mixture was stirred overnight in a sealed container. It was then cooled to 0 °C, and phosphorus oxychloride (0.0070 mL, 0.075 mmol) was added via syringe. The mixture was stirred for 3 h at 0 °C, and then tributylamine (0.060 mL, 0.25 mmol), tributylammonium pyrophosphate (0.25 mmol, 90.8 mg), and acetonitrile (stored over sieves) (0.25 mL) were added. The mixture was stirred for an additional 30 min at 0 °C, the sealed vial was then opened, and the reaction was quenched by addition of TEAB (1 M) (0.5 mL) and water (5 mL). The reaction mixture was purified on anion exchange HPLC and desalted by reverse-phase HPLC as described below. Mass and purity were confirmed by LC−MS as described vide supra. Typical yields following this procedure ranged from 5 to 15 mg of triphosphate.

**General Procedure for Purification and QC of 5′-Triphosphate Derivatives.** The triphosphate derivatives were purified by anion exchange (AX) chromatography using a 30 mm × 100 mm Mono Q column (Pharmacia) with a buffer system of 50 mM Tris, pH 8. Elution gradients were typically from 40 mM NaCl to 0.8 M NaCl in two column volumes at 6.5 mL/min. Appropriate fractions from anion exchange chromatography were collected and desalted by reverse-phase (RP) chromatography using a Luna C18 250 mm × 21 mm column (Phenomenex) with a flow rate of 10 mL/min. Elution gradients were generally from 1% to 95% methanol in 14 min at a constant concentration of 50 mM triethylammonium acetate (TEAA), pH 7. Mass spectra of the purified triphosphates were determined using on-line HPLC mass spectrometry on a Hewlett-Packard (Palo Alto, CA) MSD 1100. A Phenomenex Luna (C18(2)), 150 mm × 2 mm, plus 30 mm × 2 mm guard column, 3 mm particle size, was used for RP HPLC. A 0−50% linear gradient (15 min) of acetonitrile in 20 mM TEAA, pH 7, was performed in series with mass spectral detection in the negative ionization mode. Nitrogen gas and a pneumatic nebulizer were used to generate the electrospray. The mass range of 150−900 was sampled. Molecular masses were determined using the HP Chemstation analysis package. The purity of the purified triphosphates was determined by analytical RP and AX HPLC. RP HPLC with a Phenomonex Luna or Jupiter column (250 mm × 4.6 mm), 5 mm particle size, was typically run with a 2−70% acetonitrile gradient in 15 min in 100 mM TEAA, pH 7. AX HPLC was performed on a 5 mm × 16 mm Mono Q column (Pharmacia). Triphosphates were eluted with a gradient of 0−0.4 M NaCl at constant concentration of 50 mM Tris, pH 8. The purity of the triphosphates was generally >90%.

**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 an in situ ribonuclease protection assay (RPA), as previously described at 24 h.[14] The replicon $EC_{50}$ values are the average of at least two separate potency determination (Tables 1−4). The cytotoxicity of the nucleosides was determined by MTS at 24 h, as described in ref 14, and the $CC_{50}$ values were >50 μM (data not shown).

**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.[14] 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). Reaction mixtures were incubated at 4 °C for 27 h. RP-HPLC analysis utilized a C18 column (Vydac C218TP, 150 × 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 $\mu$L 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 was at 260 nm.

**Assay for ADA-Mediated Conversion.** Assay reactions included 5 mM Tris-HCl, pH 7.4, 5 mM potassium phosphate, 0.00625 or 1.25 U/mL adenosine deaminase (ADA, Sigma A-5168, type IX from calf spleen). Reactions were initiated with addition of 25 $\mu$M nucleoside. Reaction mixtures were incubated at room temperature for 30 min (0.006 25 U/mL ADA) or 24 h (1.25 U/mL ADA) before the enzyme was heat-inactivated (75 °C for 15 min). RP-HPLC analysis utilized a SUPELCOSIL LC-18-S column (Supelco 58931, 150 mm × 4.6 mm, 5 $\mu$M) 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 25 $\mu$L 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, 100−60% over 4.5 min); monitoring was at 254 and 280 nm. Injections of adenosine and inosine yielded linear standard curves allowing for the quantitation of substrate utilization and product formation.

**Calculations.** Nucleoside conformational preferences were calculated by molecular mechanics using the MMFFs force field and a dielectric constant, $\epsilon$, of 50. For each nucleoside, 1000 conformers were generated using the JG distance geometry program[31] and minimized to low gradient using Batch-Min.[32]

**2-Amino-6-chloro-9-(2-*C*-methyl-2,3,5-tri-*O*-benzoyl-$\beta$-D-ribofuranosyl)purine (14).** To a precooled (0 °C) solution of **13** (1.00 g, 1.72 mmol), 2-amino-6-chloropurine (0.32 g, 1.87 mmol), and 1,8-diazabicycl[5.4.0]undec-7-ene (DBU) (0.77 mL, 5.10 mmol) in anhydrous acetonitrile (20 mL) was added trimethylsilyl triflate (1.25 mL, 6.88 mmol) dropwise. The reaction mixture was heated at 65 °C for 4 h, allowed to come to room temperature, poured into saturated aqueous sodium bicarbonate (150 mL), and extracted with dichloromethane (3 × 100 mL). The combined organic phase was dried over sodium sulfate and evaporated in vacuo. The residue was purified over silica gel using hexane/ethyl acetate (7:3) as the eluent to give the desired compound (0.68 g, 63%) as a colorless foam. $^1$H NMR ($CDCl_3$): $\delta$ 8.15−7.30 (m, 16H), 6.65 (s, 1H), 6.40 (d, $J$ = 6.6 Hz, 1H), 5.35 (s, 2H), 5.08 (m, 1H), 4.84−4.73 (m, 2H), 1.60 (s, 3H). MS (ESI): calcd for $C_{32}H_{26}ClN_5O_7 + H^+$ 628.1599, found 628.0.

**2-Amino-6-chloro-9-(2-*C*-methyl-$\beta$-D-ribofuranosyl)purine (15).** A solution of **14** (0.65 g, 1.03 mmol) in saturated methanolic ammonia (15 mL) was stirred in a sealed container for 7 h. The solvent was removed, and the residue 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 compound (0.30 g, 92%) as a colorless powder. $^1$H NMR (methanol-$d_4$): $\delta$ 8.56 (s, 1H), 6.01 (s, 1H), 4.20 (d, $J$ = 8.8 Hz, 1H), 4.07−3.98 (m, 2H), 3.85 (dd, $J$ = 12.6 and 3.0 Hz, 1H), 0.97 (s, 3H). MS (ESI): calcd for $C_{11}H_{14}ClN_5O_7 + H^+$ 316.0813, found 315.9.

**2′-*C*-Methylguanosine (12).** To a solution of **15** (0.20 g, 0.63 mmol) and 2-mercaptoethanol (0.22 mL, 3.15 mmol) in methanol (20 mL) was added sodium methoxide (0.18 g, 3.15 mmol), and the mixture was heated under reflux for 7 h. The solution was cooled to room temperature and neutralized with acetic acid. The solvent was evaporated in vacuo and the residue was purified on silica gel using dichloromethane/methanol (17:3) as the eluent to give the desired compound (0.11 g, 59%) as a colorless solid. $^1$H NMR (DMSO-$d_6$): $\delta$ 10.94 (bs, 1H), 8.04 (s, 1H), 6.72 (s, 2H), 5.73 (s, 1H), 4.00−3.62 (m, 4H), 0.81 (s, 3H). HRMS (FAB): calcd for $C_{11}H_{15}N_5O_5 + H^+$ 298.1151, found 298.1149.

**6-Amino-9-(2-*C*-methyl-$\beta$-D-arabinofuranosyl)purine (19).** A mixture of chromium(VI) oxide (10.0 g, 100 mmol), pyridine (16.2 mL, 200 mmol), and acetic anhydride (9.50 mL, 100 mmol) in dichloromethane (250 mL) was stirred at 0 °C for 30 min. 3′,5′-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-adenosine **16**[23] (20.0 g, 39.2 mmol) was added, and the mixture was stirred at 0 °C for another 30 min. The mixture was diluted with ethyl acetate (1000 mL) and filtered through a pad of silica gel (200 g). The filtrate was washed with saturated aqueous sodium bicarbonate (2 × 200 mL), dried over magnesium sulfate, and evaporated in vacuo to a solid (20.5 g). To a solution of this keto derivative in dry THF (100 mL) was added methylmagnesium bromide (150 mmol in 250 mL of diethyl ether) at −78 °C. The mixture was stirred at −50 to −30 °C for 4 h, and acetone (20 mL) was then added to quench the reaction. The mixture was washed with saturated aqueous ammonium chloride (200 mL), dried over sodium sulfate, and evaporated in vacuo to a solid (15.0 g). This solid (1.00 g, 1.90 mmol) was dissolved in dichloromethane/acetonitrile (20 mL, 1:1), and triethylamine (1 mL) and $Et_3N{\cdot}3HF$ (1 mL) were added. The mixture was stirred at room temperature for 3 h and then evaporated in vacuo. The residue was purified on silica gel using dichloromethane/acetone/methanol (20:5:2) as the eluent. Fractions containing the product were pooled and evaporated in vacuo to give the desired product (0.15 g, 21% for three steps). $^1$H NMR (DMSO-$d_6$): $\delta$ 8.19 (s, 1H), 8.12 (s, 1H), 7.21 (s br, 2H), 6.15 (d, 1H, $J$ = 4.8 Hz), 5.93 (s, 1H), 5.63 (s, 1H), 5.36 (br, 1H), 3.90 (m, 1H), 3.81 (m, 1H), 3.65 (m, 2H), 1.13 (s, 3H). HRMS (FAB): calcd for $C_{11}H_{15}N_5O_4 + H^+$ 282.1202, found 282.1208.

**9-(2-*O*-Acetyl-3,5-bis-*O*-benzyl-3-*C*-methyl-$\beta$-D-ribofuranosyl)-6-chloropurine (21).** To a precooled (0 °C) mixture of **20**[24] (2.86 g, 6.67 mmol), 6-chloropurine (1.24 g, 8.00 mmol), and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (3.00 mL, 20 mmol) in anhydrous acetonitrile (27 mL) was added trimethylsilyl triflate (4.82 mL, 26.7 mmol). The mixture was heated at 60 °C for 3 h and subsequently quenched by dropwise addition of saturated aqueous sodium bicarbonate. The mixture was diluted with ethyl acetate (400 mL), washed with saturated aqueous sodium bicarbonate (2 × 300 mL), dried over sodium sulfate, and evaporated in vacuo. The crude product was purified on silica using hexane/ethyl acetate (3:1) to give the desired compound (1.60 g, 46%) as a slightly tan foam. $^1$H NMR ($CDCl_3$): $\delta$ 8.71 (s, 1H), 8.55 (s, 1H), 7.40−7.26 (m, 10H), 6.51 (d, 1H), 5.99 (d, 1H), 4.73−4.56 (m, 4H), 4.43 (m, 1H), 3.81 (dd, 1H,), 3.59 (dd, 1H), 2.04 (s, 3H), 1.51 (s, 3H). MS (ESI): calcd for $C_{27}H_{27}N_4O_5 + Na^+$ 545.1568, found 545.0.

**9-(3,5-Bis-*O*-benzyl-3-*C*-methyl-$\beta$-D-ribofuranosyl)adenine (22).** A solution of **21** (680 mg, 1.30 mmol) in a 1:1 mixture of anhydrous 1,4-dioxane and liquid ammonia (30 mL) was sealed in a stainless steel reaction vessel and heated at 80 °C for 14 h. After cooling, the vessel was opened and the solvents were allowed to evaporate. The resulting residue was dissolved in dichloromethane (200 mL), washed with brine (2 × 200 mL), dried over magnesium sulfate, and evaporated in vacuo. The crude material was purified on silica gel using dichloromethane/methanol (19:1) to give the desired compound (0.58 g, 97%) as a colorless foam. $^1$H NMR ($CDCl_3$): $\delta$ 8.22 (s, 1H), 8.03 (s, 1H), 7.40−7.26 (m, 10H), 6.21 (s br, 2H), 6.14 (d, 1H), 4.65−4.53 (m, 5H), 4.44 (m, 1H), 3.78 (dd, 1H), 3.59 (dd, 1H), 1.56 (s, 3H). MS (ESI): calcd for $C_{25}H_{27}N_5O_4 + H^+$ 462.2141, found 462.1.

**9-(3-*C*-Methyl-$\beta$-D-ribofuranosyl)adenine (23).** A mixture of **22** (0.29 g, 0.62 mmol) and ammonium formate (312 mg, 4.94 mmol) in methanol (10 mL) was sparged with argon for 10 min and then treated with 10% Pd/C (50 mg). The mixture was heated at reflux for 12 h, cooled to room temperature, and filtered through a pad of Celite. The crude material was purified on silica gel using dichloromethane/methanol (1:9), evaporated in vacuo to a pale glass, and lyophilized from water (2 × 10 mL) to give the desired compound (61 mg, 35%) as a colorless solid. $^1$H NMR (DMSO-$d_6$): $\delta$ 8.34 (s, 1H), 8.12 (s, 1H), 7.40 (s br, 2H), 5.83 (d, 1H), 4.45 (d, 1H), 3.89 (m, 1H), 3.70−3.48 (m, 2H), 1.31 (s, 3H). HRMS (FAB): calcd for $C_{11}H_{15}N_5O_4 + H^+$ 282.1202, found 282.1197.

**1-*O*-Methyl-2-*C*-methyl-2-*O*-methyl-3,5-bis-*O*-(2,4-dichlorophenylmethyl)-$\alpha$-D-ribofuranose (26).** To a solution of

**25**[26,27] (2.00 g, 4.03 mmol) and 18-crown-6 (200 mg) in dry THF (10 mL) was added powdered KOH (1.13 g, 20.2 mmol). The resulting mixture was stirred at room temperature for 60 min, and methyl iodide (0.86 g, 6.05 mmol) was added. The reaction mixture was stirred for another 3 h, the solids were filtered off, and the filtrate was concentrated in vacuo. The crude product was purified on silica gel using hexane/ethyl acetate (2:1) as the eluent to give the desired compound (2.00 g, 97%) as an oil. $^1$H NMR ($CDCl_3$): $\delta$ 7.50−7.16 (m, 6 H), 4.82 (d, 1 H, $J$ = 13.8 Hz), 4.66−4.51 (m, 4 H), 4.26 (q, 1 H), 3.65 (dd, 1 H), 3.54 (d, 2 H), 3.46 (s, 3 H), 3.40 (s, 3 H), 1.40 (s, 3 H). MS (ESI) calcd for $C_{22}H_{24}Cl_4O_5$ + $H^+$ 509.0456, found 509.5.

**1-*O*-Methyl-2-*C*-methyl-2-*O*-methyl-3,5-di-*O*-acetyl-α-D-ribofuranose (27).** A solution of compound **26** (2.00 g, 3.92 mmol) and Pd/C (10%) in a mixture of dichloromethane/methanol/acetic acid (15 mL, 1:1:1) was stirred under hydrogen at 1 atm overnight. The solids were filtered off, and the filtrate was concentrated in vacuo. The crude compound was purified on silica using dichloromethane/methanol (10:1) to give the desired compound (0.65 g, 75%). This compound (0.16 g) was treated with acetic anhydride/pyridine (4 mL, 1:1) in the presence of 4-(dimethylamino)pyridine (2 mg) at room temperature overnight. The reaction mixture was quenched with methanol and concentrated in vacuo, and the crude material was purified on silica gel using hexane/ethyl acetate (1:1) to give the desired compound as a colorless oil. $^1$H NMR ($CDCl_3$): $\delta$ 4.80 (d, $J$ = 4.8 Hz, 1H), 4.64 (s, 1H), 4.50−4.40 (m, 1H), 4.30−4.20 (m, 2H), 3.42 (s, 3H), 3.35 (s, 3H), 2.20 (s, 3H), 2.05 (s, 3H), 1.40 (s, 3H). MS (ESI) calcd for $C_{12}H_{20}O_7$ + $H^+$ 299.1107, found 299.1.

**1-*O*-Acetyl-2-*C*-methyl-2-*O*-methyl-3,5-di-*O*-acetyl-D-ribofuranose (28).** To a precooled (0 °C) solution of compound **27** (0.23 g, 0.74 mmol) in anhydrous acetic anhydride (3.12 mL) was added concentrated sulfuric acid (0.071 mL) in anhydrous acetic anhydride (3.12 mL) dropwise. The reaction mixture was stirred at 0 °C for 1 h. The mixture was neutralized with cold saturated aqueous sodium bicarbonate solution and extracted with ethyl acetate (3 × 50 mL). The organic phase was washed with saturated aqueous sodium bicarbonate (2 × 100 mL) and then with brine (100 mL), dried over magnesium sulfate, and evaporated in vacuo. The crude product was purified on silica gel using hexane/ethyl acetate (4:1) as the eluent to give the desired compound (0.22 g, 98%) as an anomeric mixture. $^1$H NMR ($CDCl_3$): $\delta$ 6.99 (s, 1H), 6.20 (s, 1H), 5.54−5.47 (m, 1H), 5.36 (d, $J$ = 4.0 Hz, 1H), 5.19 (d, $J$ = 6.7 Hz, 1H), 4.54 (dd, 1H), 4.40−4.07 (m, 4H), 3.38 (s, 3H), 3.35 (s, 3H), 2.17 (s, 3H), 2.15 (s, 3H), 2.13 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 1.30 (s, 3H), 1.27 (s, 3H). MS (ESI) calcd for $C_{13}H_{20}O_8$ + $H^+$ 327.1056, found 327.2.

**2′-*C*-Methyl-2′-*O*-methyladenosine (29).** To a precooled (0 °C) mixture of **28** (0.25 g, 0.76 mmol), 6-chloropurine (0.11 g, 0.70 mmol), and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.29 mL) in anhydrous acetonitrile (6.0 mL) was added trimethylsilyl triflate (0.42 mL, 2.18 mmol). The reaction mixture was then stirred for 4 h at 65 °C, evaporated in vacuo, and purified on silica using hexane/ethyl acetate (1:1) as the eluent to give the desired intermediate (0.17 g, 56%) as an anomeric mixture. This compound was treated with methanolic ammonia (4 mL) at room temperature overnight. The reaction mixture was evaporated in vacuo and purified on silica gel using dichloromethane/methanol (5:1) as the eluent to give the desired compound **29** (7.5 mg), the α anomer (3.5 mg), and a mixture of compound **29** and the α anomer (21 mg) in 32% total yield as white solids. The overall α/$\beta$ anomeric ratio was 2:1.

**α-Anomer.** $^1$H NMR (methanol-$d_4$): $\delta$ 8.26 (s, 1H), 8.18 (s, 1H), 6.18 (s, 1H), 4.30−4.18 (m, 1H), 4.06 (d, 1H), 3.86 (dd, 1H), 3.65 (dd, 1H), 3.15 (s, 3H), 1.56 (s, 3H). MS (ESI): calcd for $C_{24}H_{34}N_{10}O_8$ + $Na^+$ (2M + $Na^+$) 613.2459, found 612.9.

**$\beta$-Anomer.** $^1$H NMR (methanol-$d_4$): $\delta$ 8.59 (s, 1H), 8.20 (s, 1H), 6.27 (s, 1H), 4.21 (d, 1 H), 4.07−3.99 (m, 2 H), 3.89−3.82 (m, 1H), 3.54 (s, 3H), 0.95 (s, 3H). HRMS (FAB): calcd for $C_{12}H_{17}N_5O_4$ + $H^+$ 296.1359, found 296.1359.

**1-*O*-Methyl-2-*C*-ethyl-3,5-bis-(2,4-dichlorophenylmethyl)-α-D-ribofuranose (31).** To a precooled (0 °C) suspension of Dess−Martin periodinane (10.1 g, 23.0 mmol) in anhydrous dichloromethane (50 mL) was added a solution of **30** (3.70 g, 7.60 mmol) in dichloromethane (50 mL) dropwise. The reaction mixture was then stirred at room temperature for 48 h. The mixture was diluted with diethyl ether (200 mL) and poured into a chilled solution of $Na_2S_2O_3$·$5H_2O$ (18 g) in saturated aqueous sodium bicarbonate (150 mL). The organic phase was washed with saturated aqueous sodium bicarbonate (100 mL), then water (150 mL), and then brine (150 mL). The organic phase was dried over sodium sulfate and evaporated in vacuo to give the keto derivative, which was used for the next reaction without purification. To diethyl ether (120 mL) at −78 °C was slowly added EtMgBr (3.0 M, 12.6 mL) followed by the keto derivative (obtained vide infra) in diethyl ether (75 mL). The reaction mixture was stirred at −78 °C for 30 min and then allowed to come to −15 °C and stirred for 4 h. The reaction mixture was poured into saturated aqueous ammonium chloride. The organic phase was washed with brine, dried over sodium sulfate and evaporated in vacuo. The residue was purified over silica gel using hexane/ethyl acetate (9:1) as the eluent to give the desired compound (1.30 g, 34%). $^1$H NMR ($CDCl_3$): $\delta$ 7.46−7.17 (m, 6H), 4.82−4.55 (m, 5H), 4.15 (q, $J$ = 4.4, 8.8 Hz, 1H), 3.64 (d, $J$ = 4.4 Hz, 2H), 3.45 (s, 3H), 3.26 (s, 1H), 1.62 (m, 2H), 0.99 (t, $J$ = 7.2 Hz, 3H). MS (FAB): calcd for $C_{22}H_{24}Cl_4O_5$ + $Li^+$ 517.17, found 517.10

**1-*O*-Methyl-2-*C*-ethyl-2-*O*-benzoyl-α-D-ribofuranoside (32).** To a solution of 4-(dimethylamino)pyridine (DMAP) (0.34 g, 2.80 mmol) and triethylamine (4.40 mL) in dichloromethane (10 mL) was added benzoyl chloride (0.64 mL, 5.50 mmol) followed by **31** (0.65 g, 1.20 mmol) in dichloromethane (10 mL). The reaction mixture was heated at 50 °C for 48 h. The solution was cooled to room temperature and poured into a solution of diethyl ether (150 mL) and saturated aqueous sodium bicarbonate (150 mL). The organic phase was washed with aqueous sodium bicarbonate (2 × 100 mL) and then with brine (100 mL), dried over sodium sulfate, and evaporated in vacuo. The crude product was purified on silica gel using ethyl acetate/hexane (1:9) as the eluent to give the desired compound (0.70 g, 95%). A suspension of this benzoate derivative and Pd/C (10%) (0.70 g) in methanol (5 mL) and acetic acid (2 mL) was stirred under hydrogen at 1 atm overnight. The solution was filtered, evaporated in vacuo, and coevaporated from toluene. The residue was purified over silica gel using ethyl acetate/hexane (3:7) as the eluent to give the desired compound (0.11 g, 32%) as a colorless oil. $^1$H NMR ($CDCl_3$): $\delta$ 8.07−8.02 (m, 2H), 7.61−7.44 (m, 3H), 4.92 (d, $J$ = 4.8 Hz, 1H), 4.67 (s, 1H), 4.19 (q, $J$ = 4.6, 8.8 Hz, 1H), 3.88 (m, 2H), 3.49 (s, 3H), 3.11 (s, 1H), 2.31 (t, 1H), 1.74 (m, 2H), 1.03 (t, $J$ = 7.2 Hz, 3H). MS (FAB): calcd for $C_{15}H_{21}O_6$ + $Li^+$ 303.14, found 303.20.

**1-*O*-Methyl-2-*C*-ethyl-2,5-di-*O*-benzoyl-α-D-ribofuranose (33).** To a solution of 4-(dimethylamino)pyridine (DMAP) (0.10 g, 0.82 mmol) and triethylamine (0.80 mL) in dichloromethane (5 mL) was added benzoyl chloride (0.26 mL, 2.20 mmol) followed by **32** in dichloromethane (5 mL). The reaction mixture was stirred at room temperature for 3 h. This solution was poured into a mixture of diethyl ether (100 mL) and saturated aqueous sodium bicarbonate (100 mL). The organic phase was washed with aqueous sodium bicarbonate (100 mL) and brine (100 mL), dried over sodium sulfate, and evaporated in vacuo. The residue was purified on silica gel using hexane/ethyl acetate (4:1) as the eluent to give the desired compound (0.16 g, 94%). $^1$H NMR ($CDCl_3$): $\delta$ 8.09−8.01 (m, 4H), 7.60−7.37 (m, 6H), 5.11 (d, $J$ = 4.6 Hz, 1H), 4.75−4.45 (m, 5H), 3.52 (s, 3H), 1.78 (m, 2H), 1.05 (t, $J$ = 7.4 Hz, 3H). MS (FAB): calcd for $C_{22}H_{25}O_7$ + $Li^+$ 407.17, found 407.20

**1-*O*-Acetyl-2-*C*-ethyl-2,5-di-*O*-benzoyl-3-*O*-acetyl-D-ribofuranose (34).** To a solution of the dibenzoyl derivative **33** in acetic acid (5 mL) were added acetic anhydride (1.0 mL) and sulfuric acid (0.2 mL) at 0 °C. The reaction mixture was stirred at room temperature overnight. The solution was poured into ice and extracted with dichloromethane (3 × 40 mL). The combined organic phase was washed with saturated aqueous sodium bicarbonate (2 × 50 mL), dried over sodium sulfate, and evaporated in vacuo. The residue was purified over

silica gel using hexane/ethyl acetate (3:1) as the eluent to give the desired compound (0.085 g, 51%) as an anomeric mixture. $^1$H NMR ($CDCl_3$): $\delta$ 8.09−7.34 (m, 10 H), 6.61 (s, 1H), 6.51 (s, 1H), 5.80 (d, $J$ = 7.8 Hz, 1H), 5.51 (d, $J$ = 3.2 Hz, 1H), 4.73−4.38 (m, 6H), 2.41−2.02 (m, 4H), 2.19 (s, 3H), 2.16 (s, 3H), 1.99 (s, 1H), 1.92 (s, 3H). LRMS (FAB): calcd for $C_{25}H_{27}O_9 + Li^+$ 477.17, found 477.20

**6-Amino-9-(2-*C*-ethyl-β-D-ribofuranosyl)purine (36).** To a precooled (0 °C) solution of **34** (0.085 g, 0.18 mmol), 6-chloropurine (0.042 g, 0.27 mmol), and 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) (0.08 mL, 0.55 mmol) in acetonitrile (8 mL) was added trimethylsilyl triflate (0.13 mL, 0.76 mmol). The reaction mixture was stirred at 50 °C for 1.5 h. The solution was allowed to come to room temperature, poured into saturated aqueous sodium bicarbonate (100 mL), and extracted with ethyl acetate (100 mL). The organic phase was washed with aqueous sodium bicarbonate (2 × 50 mL) and brine (100 mL), dried over sodium sulfate, and evaporated in vacuo. The residue was purified on silica gel using hexane/ethyl acetate (7:3) to give the desired compound as a colorless powder. A solution of this compound (**35**) in dioxane (2 mL) and liquid ammonia (8 mL) was heated in a Parr bomb at 60 °C overnight. The solution was evaporated to dryness and the residue was purified on silica gel using dichloromethane/methanol (9:1) as the eluent to give the desired compound (13.0 mg, 16%). $^1$H NMR (methanol-$d_4$): $\delta$ 8.48 (s, 1H), 8.19 (s, 1H), 6.12 (s, 1H), 4.38 (d, $J$ = 8.8 Hz, 1H), 4.08−3.96 (m, 2H), 3.85 (dd, $J$ = 3.2, 12.6 Hz, 1H), 1.44−1.06 (m, 2H), 0.78 (t, $J$ = 7.4 Hz, 3H). HRMS (FAB): calcd for $C_{12}H_{17}N_5O_4$ 296.1358, found 296.1355.

**2′-*C*-Methylinosine (38).** A solution of **37** (0.02 g, 0.067 mmol) in 1 N aqueous sodium hydroxide (2 mL) was heated at reflux for 2 h. The mixture was cooled, neutralized with HCl (1 N, aqueous), and evaporated in vacuo. The residue was purified on silica gel using methanol/dichloromethane (1:9 through 1:4) as the eluent. Fractions containing the desired compound were pooled and evaporated in vacuo to give the desired product (8.0 mg, 42.3%) as a colorless powder after freeze-drying. $^1$H NMR (DMSO-$d_6$): $\delta$ 12.15 (s br, 1H), 8.40 (s, 1H), 8.01 (s, 1H), 5.85 (s, 1H), 5.15−5.21 (overlapping t, d, and s, 3H), 3.65−4.13 (overlapping m, 4H), 0.71 (s, 3H).

**(2-*C*-Methyl-β-D-ribofuranosyl)purine (39).** A mixture of **37** (0.020 g, 0.067 mmol), triethylamine (0.1 mL), and Pd (10% on carbon) (0.01 g) in methanol (5.0 mL) was stirred under hydrogen overnight. The mixture was filtered through Celite, evaporated in vacuo, and purified on silica gel using methanol/dichloromethane (1:9) as the eluent. Fractions containing the desired compound were pooled and evaporated in vacuo to give the desired product (12 mg, 67%) as a colorless powder after freeze-drying. $^1$H NMR (acetonitrile-$d_3$): $\delta$ 9.10 (s, 1H), 8.93 (s, 1H), 8.70 (s, 1H), 6.19 (s, 1H), 4.01 (m, 2H), 3.66−4.02 (overlapping m, 1H), 3.83 (dd, $J$ = 13.0 and 3.4 Hz, 1H), 0.88 (s, 3H). HRMS (FAB): calcd for $C_{10}H_{15}N_4O_4 + H^+$ 268.1126, found 268.1129.

**2,6-Diamino-9-(2-*C*-methyl-β-D-ribofuranosyl)purine (40).** To **15** (0.10 g, 0.32 mmol) was added ammonium hydroxide (5 mL), and the resulting mixture was heated to 80 °C overnight in a sealed container. The mixture was evaporated in vacuo and purified on silica using methanol/dichloromethane (1:9 through 1:4) as the eluent. Fractions containing the desired product were pooled and evaporated in vacuo to give the desired product (0.054 g, 57.5%) as a colorless powder after freeze-drying. $^1$H NMR (methanol-$d_4$): $\delta$ 8.13 (s, 1H), 5.91 (s, 1H), 4.19 (d, $J$ = 8.8 Hz, 1H), 4.01 (m, 2H), 3.83 (dd, $J$ = 13.0 and 3.4 Hz, 1H), 0.94 (s, 3H). HRMS (FAB): calcd for $C_{11}H_{17}N_6O_4 + H^+$ 297.1311, found 297.1305.

**2-Amino-9-(2-*C*-methyl-β-D-ribofuranosyl)purine (41).** A mixture of **15** (0.02 g, 0.064 mmol), triethylamine (0.1 mL), and Pd/C (0.01 g) in methanol (5.0 mL) was stirred under hydrogen overnight. The mixture was filtered through Celite, evaporated in vacuo, and purified on silica gel using methanol/dichloromethane (1:9) as the eluent. Fractions containing the desired compound were pooled and evaporated in vacuo to give the desired product (0.014 g, 78%) as a colorless powder after freeze-drying. $^1$H NMR (DMSO-$d_6$): 8.60 (s, 1H), 8.40 (s, 1H), 6.58 (s br, 1H), 5.25 (d, 1H), 5.16 (t, 1H), 5.16 (s, 1H), 3.60−4.06 (overlapping m, 4H), 0.81 (s, 3H). HRMS (FAB): calcd for $C_{11}H_{15}N_5O_4 + H^+$ 282.1202, found 282.1199.

## Appendix

**Abbreviations.** ADA, adenosine deaminase; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DMAP, 4-(dimethylamino)pyridine; HCV, hepatitis C virus; NS5B, nonstructural protein 5B; RdRp, RNA-dependent RNA polymerase; NNI, non-nucleoside inhibitor; NI, nucleoside inhibitor; HIV, human immunodeficiency virus; NTP, nucleoside triphosphate, AX, anion exchange; RP, reverse phase; HPLC, high-performance liquid chromatography; RPA, ribonuclease protection assay; PNP, purine nucleoside phosphorylase; TIPDS dichloride, 1,3-dichloro-1,1,3,3,-tetraisopropyldisiloxane.

## References


(1) Hwang, S. B.; Park, K.-J.; Kim, Y.-S.; Sung, Y. C.; Lai, M. M. C. Hepatitis C virus NS5B protein is a membrane-associated phosphoprotein with a predominantly perinuclear localization. *Virology* **1997**, *227*, 439−446.

(2) Behrens, S.-E.; Tomei, L.; De Francesco, R. Identification and properties of the RNA-dependent RNA polymerase of hepatitis C virus. *EMBO J.* **1996**, *15*, 12−22.

(3) Takamizawa, A.; Mori, C.; Fuke, I.; Manabe, S.; Murakami, S.; Fujita, J.; Onishi, E.; Andoh, T.; Yoshida, I.; Okayama, H. Structure and organization of the hepatitis C virus genome isolated from human carriers. *J. Virol.* **1991**, *65*, 1105−1113.

(4) Colarusso, S.; Gerlach, B.; Koch, U.; Muraglia, E.; Conte, I.; Stansfield, I.; Matassa, V. G.; Narjes, F. Evolution, synthesis and SAR of tripeptide α-ketoacid inhibitors of the hepatitis C virus NS3/NS4A serine protease. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 705−708.

(5) Narjes, F.; Koehler, K. F.; Koch, U.; Gerlach, B.; Colarusso, S.; Steinkuhler, C.; Brunetti, M.; Altamura, S.; De Francesco, R.; Matassa, V. G. A designed P1 cysteine mimetic for covalent and non-covalent inhibitors of HCV NS3 protease. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 701−704.

(6) Steinkuhler, C.; Koch, U.; Narjes, F.; Matassa, V. G. Hepatitis C virus serine protease inhibitors: current progress and future challenges. *Curr. Med. Chem.* **2001**, *8*, 919−932.

(7) Llinas-Brunet, M.; Bailey, M.; Fazal, G.; Goulet, S.; Halmos, T.; Laplante, S.; Maurice, R.; Poirier, M.; Poupart, M.-A.; Thibeault, D.; Wernic, D.; Lamarre, D. Peptide-based inhibitors of the hepatitis C virus serine protease. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1713−1718.

(8) Llinas-Brunet, M.; Bailey, M.; Deziel, R.; Fazal, G.; Gorys, V.; Goulet, S.; Halmos, T.; Maurice, R.; Poirier, M.; Poupart, M.-A.; Rancourt, J.; Thibeault, D.; Wernic, D.; Lamarre, D. Studies on the C-terminal of hexapeptide inhibitors of the hepatitis C virus serine protease. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2719−2724.

(9) Lamarre, D.; Anderson, P. C.; Balley, M.; Beaulieu, P.; Bolger, G.; Bonneau, P.; Bos, M.; Cameron, D. R.; Cartier, M,; Cordingley, M. G.; Faucher A.-M.; Goudreau, N.; Kawai, S. H.; Kukolj, G.; Lagacé, l.; Laplante, S. R.; Narjes, H.; Poupart, M.-A.; Rancourt, G.; Sentjens, R. E.; St. George, R.; Simoneau, B.; Stelnmann, G.; Thibeault, D.; Tsantrzos, Y. S.; Weldon, S. M.; Yong, C.-L.; Llinas-Brunet, M. *Nature* **2003**, *426*, 186−189.

(10) Chan, L.; Reddy, T. J.; Proulx, M.; Das, S. K.; Pereira, O.; Wang, W.; Siddiqui, A.; Yannopoulos, C. G.; Poisson, C.; Turcotte, N.; Drouin, A.; Alaoui-Ismaili, M. H.; Bethell, R.; Hamel, M.; L'Heureux, L.; Bilimoria, D.; Nguyen-Ba, N. Identification of N,N-Disubstituted Phenylalanines as a Novel Class of Inhibitors of Hepatitis C NS5B Polymerase. *J. Med. Chem.* **2003**, *46*, 1283−1285.

(11) Wang, M.; Ng, K. K. S.; Cherney, M. M.; Chan, L.; Yannopoulos, C. G.; Bedard, J.; Morin, N.; Nguyen-Ba, N.; Alaoui-Ismaili, M. H.; Bethell, R. C.; James, M. N. G. Non-nucleoside Analogue Inhibitors Bind to an Allosteric Site on HCV NS5B Polymerase. *J. Biol. Chem.* **2003**, *278*, 9489−9495.

(12) Dhanak, D.; Duffy, K. J.; Johnston, V. K.; Lin-Goerke, J.; Darcy, M.; Shaw, A. N.; Gu, B.; Silverman, C.; Gates, A. T.; Nonnemacher, M. R.; Earnshaw, D. L.; Casper, D. J.; Kaura, A.; Baker, A.; Greenwood, C.; Gutshall, L. L.; Maley, D.; DelVecchio, A.; Macarron, R.; Hofmann, G. A.; Alnoah, Z.; Cheng, H.-Y.; Chan, G.; Khandekar, S.; Keenan, R. M.; Sarisky, R. T. Identification and Biological Characterization of Heterocyclic Inhibitors of the Hepatitis C Virus RNA-Dependent RNA Polymerase. *J. Biol. Chem.* **2002**, *277*, 38322−38327.

(13) Gu, B.; Johnston, V. K.; Gutshall, L. L.; Nguyen, T. T.; Gontarek, R. R.; Darcy, M. G.; Tedesco, R.; Dhanak, D.; Duffy, K. J.; Kao, C. C.; Sarisky, R. T**.** Arresting Initiation of Hepatitis C Virus RNA Synthesis Using Heterocyclic Derivatives. *J. Biol. Chem.* **2003**, *278*, 16602−16607.

(14) Carroll, S. S.; Tomassini, J. E.; Bosserman, M.; Getty, K.; Stahlhut, M. W.; Eldrup, A. B.; Bhat, B.; Hall, D.; Simcoe, A. L.; LaFemina, R.; Rutkowski, C. A.; Wolanski, B.; Yang, Z.; Migliaccio, G.; De Francesco, R.; Kuo, L. C.; MacCoss, M.; Olsen, D. B. Inhibition of Hepatitis C Virus RNA Replication by 2′-Modified Nucleoside Analogs. *J. Biol. Chem.* **2003**, *278*, 11979−11984.

(15) Shim, J.; Larson, G.; Lai, V.; Naim, S.; Wu, J. Z. Canonical 3′-deoxyribonucleotides as chainterminator for HCV NS5B RNA-dependent RNA polymerase. *Antiviral Res.* **2003**, 243−251.

(16) Migliaccio, G.; Tomassini, J. E.; Carroll, S. S.; Tomei, L.; Altamura, S.; Bhat, B.; Bartholomew, L.; Bosserman, M.; Ceccacci, A.; Colwell, L. F.; Cortese, R.; De Francesco, R.; Eldrup, A. B.; Getty, K.; Hou, W. S.; LaFemina, R.; Ludmere, S. W.; MacCoss, M.; MacMasters, D. R.; Stahlhut, M. W.; Olsen, D. B.; Hazuda, D. J..; Flores, O. Identification and validation of ribonucleoside analogues as hepatitis C antiviral agents. *J. Biol. Chem.* **2003**, *278*, 49164−49170.

(17) Crotty, S.; Cameron, C. E.; Andino, R. RNA virus error catastrophe: direct molecular test by using ribavirin. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 6895−6900.

(18) Pankiewicz, K. W.; Krzeminski, J.; Watanabe, K. A. Nucleosides. 164. Studies directed toward the synthesis of 2′-deoxy-2′-substituted arabino nucleosides. 10. Synthesis of 2′-*β*-fluoro- and 3′-α-fluoro-substituted guanine nucleosides. Effect of sugar conformational shifts on nucleophilic displacement of the 2′-hydroxy and 3′-hydroxy group with DAST. *J. Org. Chem.* **1992**, *57*, 7315−7321.

(19) Kawasaki, A. M.; Casper, M. D.; Freier, S. M.; Lesnik, E. A.; Zounes, M. C.; Cummins, L. L.; Gonzalez, C.; Cook, P. D. Uniformly modified 2′-deoxy-2′-fluoro phosphorothioate oligonucleotides as nuclease-resistant antisense compounds with high affinity and specificity for RNA targets. *J. Med. Chem.* **1993**, *36*, 831−841.

(20) Franchetti, P.; Cappellacci, L.; Marchetti, S.; Trincavelli, L.; Martini, C.; Mazzoni, M. R.; Lucacchini, A.; Grifantini, M. 2′-C-Methyl Analogs of Selective Adenosine Receptor Agonists: Synthesis and Binding Studies. *J. Med. Chem.* **1998**, *41*, 1708−1715.

(21) Harry-O'kuru, R. E.; Smith, J. M.; Wolfe, M. S. A Short, Flexible Route toward 2′-C-Branched Ribonucleosides. *J. Org. Chem.* **1997**, *62*, 1754−1759.

(22) Samano, V.; Robins, M. J. Nucleic acid related compounds. 60. Mild periodinane oxidation of protected nucleosides to give 2′- and 3′-ketonucleosides. The first isolation of a purine 2′-deoxy-3′-ketonucleoside derivative. *J. Org. Chem.* **1990**, *55*, 5186−5188.

(23) Markiewicz, W. T.; Wiewiorowski, M. A new type of silyl protecting groups in nucleoside chemistry. *Nucleic Acids Res., Spec. Publ.* **1978**, *4*, 185−188.

(24) Schmit, C.; Bevierre, M.-O.; De Mesmaeker, A.; Altmann, K.-H. The effects of 2′- and 3′-alkyl substituents on oligonucleotide hybridization and stability. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1969−1974.

(25) Koole, L. H.; Buck, H. M.; Vial, J. M.; Chattopadhyaya, J. Synthesis and conformation of 9-(3′-C-methyl-*β*-D-xylo-furanosyl)adenine and 3′-C-methyladenosine, two sugar-methylated nucleoside analogs. *Acta Chem. Scand.* **1989**, *43*, 665−669.

(26) Carroll, S. S.; Lafemina, R. L.; Hall, D. L.; Himmelberger, A. L.; Kuo, L. C.; Maccoss, M.; Olsen, D. B.; Rutkowski, C. A.; Tomassini, J. E.; An, H.; Bhat, B.; Bhat, N.; Cook, P. D.; Eldrup, A. B.; Guinosso, C. J.; Prhavc, M.; Prakash, T. P. Preparation of nucleoside derivatives as inhibitors of RNA-dependent RNA viral polymerase. PCT Int. Appl. WO 02/57425, 2002; 235 pp.

(27) Carroll, S. S.; Maccoss, M.; Olsen, D. B.; Bhat, B.; Bhat, N.; Cook, P. D.; Eldrup, A. B.; Prakash, T. P.; Prhavc, M.; Song, Q. Preparation of nucleoside derivatives as inhibitors of RNA-dependent RNA viral polymerase. PCT Int. Appl. WO 02/57287, 2002; 85 pp.

(28) Plavec, J.; Tong, W.; Chattopadhyaya, J. How do the gauche and anomeric effects drive the pseudorotational equilibrium of the pentofuranose moiety of nucleosides? *J. Am. Chem. Soc.* **1993**, *115*, 9734−9746.

(29) Ford, H., Jr.; Dai, F.; Mu, L.; Siddiqui, M. A.; Nicklaus, M. C.; Anderson, L.; Marquez, V. E.; Barchi, J. J. Adenosine Deaminase Prefers a Distinct Sugar Ring Conformation for Binding and Catalysis: Kinetic and Structural Studies. *Biochemistry* **2000**, *39*, 2581−2592.

(30) Seela, F.; Muth, H. P.; Bindig, U. Synthesis of 6-substituted 7-carbapurine 2′,3′-dideoxynucleosides. Solid−liquid phase-transfer glycosylation of 4-chloropyrrolo[2,3-*d*]pyrimidine and deoxygenation of its 2′-deoxyribofuranoside. *Synthesis* **1988**, 670−674.

(31) Kearsley, S. K. Unpublished in-house distance geometry program. In *Distance Geometry and Molecular Conformation*, 2nd ed.; Crippen, C. M., Havel, T. F. Eds.; Research Studio Press, Wiley: New York.

(32) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. MacroModel−an integrated software system for modeling organic and bioorganic molecules using molecular mechanics. *J. Comput. Chem.* **1990**, *11*, 440−467.

JM030424E