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Synthesis and HCV inhibitory properties of 9-deaza- and 7,9-dideaza-7-oxa-2′-*C*-methyladenosine

Gabor Butora,[a,*] David B. Olsen,[b] Steven S. Carroll,[b] Daniel R. McMasters,[a] Christoph Schmitt,[a] Joseph F. Leone,[a] Mark Stahlhut,[b] Christine Burlein[b] and Malcolm MacCoss[a]

[a]*Department of Medicinal Chemistry, Merck Research Laboratories, Rahway, NJ 07065, USA*
[b]*Department of Antiviral Research, Merck Research Laboratories, West Point, PA 19486, USA*

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Abstract—As a part of an ongoing medicinal chemistry effort to identify inhibitors of the Hepatitis C Virus RNA replication, we report here the synthesis and biological evaluation of 9-deaza- and 7,9-dideaza-7-oxa-2′-*C*-methyladenosine. The parent 2′-*C*-methyladenosine shows excellent intracellular inhibitory activity but poor pharmacokinetic profile. Replacement of the nucleoside-defining 9-N of 2′-*C*-methyladenosine with a carbon atom was designed to yield metabolically more stable C-nucleosides. Modifications at position 7 were designed to exploit the importance of the hydrogen bond accepting properties of this heteroatom in modulating the adenosine deaminase (ADA) mediated 6-N deamination. 7-Oxa-7,9-dideaza-2′-*C*-methyladenosine was found to be a moderately active inhibitor of intracellular HCV RNA replication, whereas 9-deaza- 2′-*C*-methyladenosine showed only weak activity despite excellent overlap of both of the synthesized target compounds with 2′-*C*-methyladenosine's three dimensional structure. Position 7 of the nucleobase proved to be an effective handle for modulating ADA-mediated degradation, with the rate of degradation correlating with the hydrogen-bonding properties at this position.

1. Introduction

Hepatitis C virus (HCV) infection is a leading cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma.[1] At the present time the therapeutic options are limited to subcutaneous administration of often poorly tolerated pegylated interferon-α in combination with the orally dosed nucleoside analog ribavirin.[2,3]

Recent advances in the molecular virology of HCV[4] have led to the identification of a number of antiviral molecular targets such as interference with viral polypeptide processing, RNA regulatory elements, and RNA replication.[5,6] Valopicitabine (NM283), currently in development by Idenix, is an example of an NS5B RNA-dependent RNA polymerase inhibitor.[7]

Modifications in the vicinity of the 3′-hydroxyl of the ribose in natural ribonucleosides can produce effective RNA chain terminators.[8] For example, replacement of the 2′-hydrogen of natural ribonucleosides with a methyl group yields compounds with excellent chain-terminating properties.[9] 2′-*C*-methyladenosine (**1**) (Fig. 1) was found to inhibit HCV RNA replication without significant toxicity.[9,10] Unfortunately, the poor pharmacokinetic

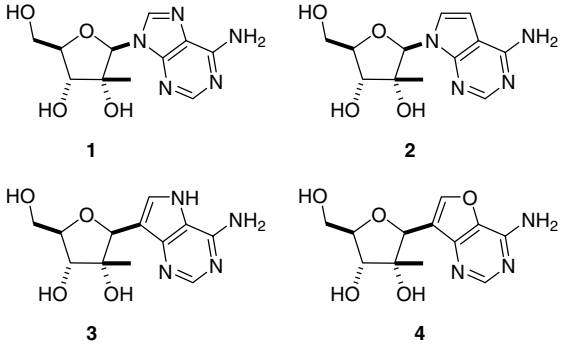

Figure 1. C_2'-Methyl adenosines.

Keywords: Nucleosides; Hepatitis C; Hepatitis C virus; RNA polymerase; Adenosine deaminase.
* Corresponding author. Tel.: +1 732 594 8563; fax: +1 732 594 9556; e-mail: gabor_butora@merck.com

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properties (mainly its rapid metabolic degradation) severely limit its potential therapeutic use. Systematic investigation in this series has shown that replacement of the 7-N of the heterobase with a carbon atom (2′-*C*-methyl-7-deaza-adenosine, **2**, Fig. 1) yields a compound with even higher potency and much improved pharmacokinetic properties.[11,12] The absence of 7-N greatly contributes to its metabolic stability, as the major pathway of adenosine degradation, 6-NH_2 deamination, requires the presence of a hydrogen bond accepting heteroatom at position 7 of the heterobase.[13]

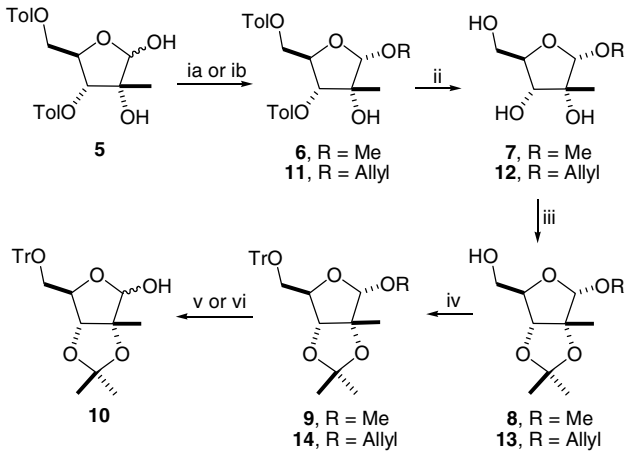
In order to obtain further structure–activity information, 2′-*C*-methyl-9-deaza-adenosine (**3**, Fig. 1), a structural isomer of **2**, was synthesized. The replacement of 9-N with a carbon atom should stabilize the molecule and for tautomeric reasons the character of the 7-N changes from a hydrogen bond acceptor to donor. Given the role 7-N plays in 6-NH_2 deamination,[13] 2′-*C*-methyl-9-deaza-adenosine should be metabolically more stable than 2′-*C*-methyl-adenosine (**1**). In the case of 2′-*C*-methyl-7,9-dideaza-7-oxa-adenosine (**4**, Fig. 1), the replacement of 9-N for carbon was designed to stabilize the molecule, while the swap of 7-N for oxygen changes the character of the 7-heteroatom from donor to an a hydrogen bond acceptor.

2. Chemistry

Although published procedures[14,15] for preparation of substituted 9-deazaadenosine analogs allowed for a direct glycosylation of a suitably substituted heterocycle (e.g., guanine or 2,4-dichloro-5*H*-pyrrolo[3,2-*d*]pyrimidine) under Vorbruggen conditions, a well-established and more flexible stepwise approach was followed.[16,17]

3′,5′-*O*-(4-Methylbenzoyl)-2′-*C*-methyl-ribose (**5**) could be conveniently synthesized following procedure relying on a base-catalyzed intramolecular ester migration in acylated carbohydrates.[18,19] Our initial attempts at 1′-*C*-functionalization of 2′,3′,5′-(4-methylbenzoyl)-protected ribose failed and a synthetic sequence yielding 2′-*C*-methyl ribose equipped with base-stable protecting groups had to be developed, Scheme 1.

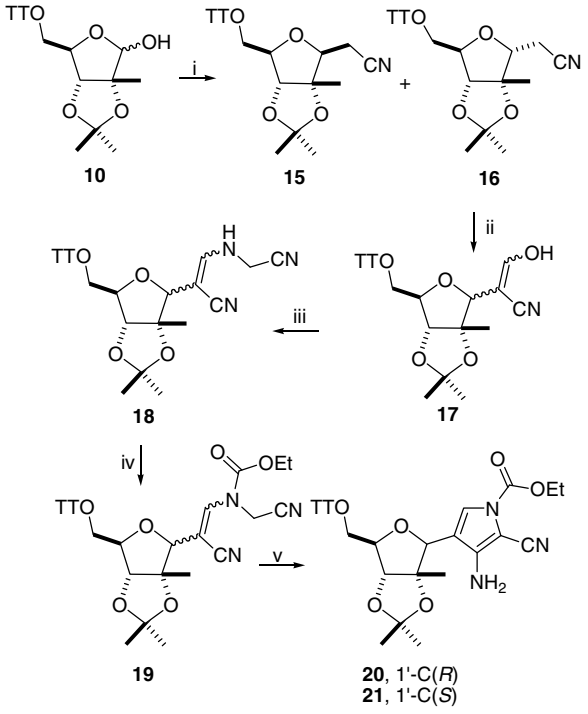
According to this sequence, a pyridinium *para*-toluenesulfonate catalyzed glycosylation of **5** performed initially with methyl alcohol afforded the glycoside **6**. The ester groups were removed by a sodium methoxide catalyzed trans-esterification, the vicinal 2′,3′-diol in **7** converted to an acetonide (**8**), and the 5′-primary hydroxyl was protected with a trityl group (**9**). Even though the usual conditions used to cleave glycosides (such as described in ref. 20) followed by base hydrolysis did produce the desired hemiacetal **10**, a partial cleavage of the 5′-*O*-trityl group induced by the acidic conditions rendered the reaction poorly reproducible. Therefore, the 1′-*O*-methyl group was replaced with a group the removal of which does not require the scission of the glycosidic 1′-*C*–O bond, such as allyl.[21,22] The above sequence could be easily modified (intermediates **11**–**14**) to yield the respective 1′-*O*-allyl glycoside **14**. A number of



Scheme 1. Reagents and conditions: (i) a—methanol or b—allyl alcohol, PPTSA, 100 °C, 24 h; (ii) MeONa, MeOH, rt, 2 h; (iii) 2,2-dimethoxypropane, pTSA, rt, 2 h; (iv) triphenylchloromethane, Py, 60 °C, 18 h; (v) R = Me; a—AcOH, Ac_2O, DCM, cat.H_2SO_4, 0 °C, 18 h; b—aq. NaOH, 15 min; (vi) R = Allyl, 1,3-bis(diphenylphosphino)propane nickel (II) dichloride, Dibal-H, diethyl ether, 0 °C, then rt 60 min.

approaches for effective removal of the 1′-*O*-allyl protecting group in **14** were tested,[23–25] and the most satisfactory procedure was found to be the nickel (II)-catalyzed Dibal-H-mediated reductive cleavage developed by Taniguchi.[26]

When 2′-*C*-methyl-2′,3′-*O*-(1-methylethylidene)-5′-*O*-trityl-D-ribofuranose (**10**) was subjected to the Moffatt procedure,[25] intermediates **15** and **16** were obtained in excellent yield (Scheme 2). The observed diastereoiso-

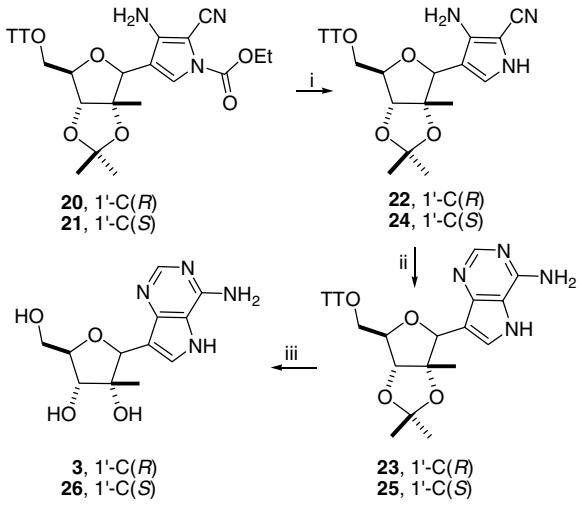


Scheme 2. Reagents and conditions: (i) diethyl cyanomethylphosphorane, NaH, DME, 0 °C then rt, 4 h; (ii) LDA, MeOCHO, THF, −78 °C, 2 h then rt 20 min; (iii) H_2NCH_2CN, NaOAc, MeOH, rt, 72 h; (iv) EtOCOCl, DBU, DCM, rt; (v) DBU, DCM, rt, 24 h.

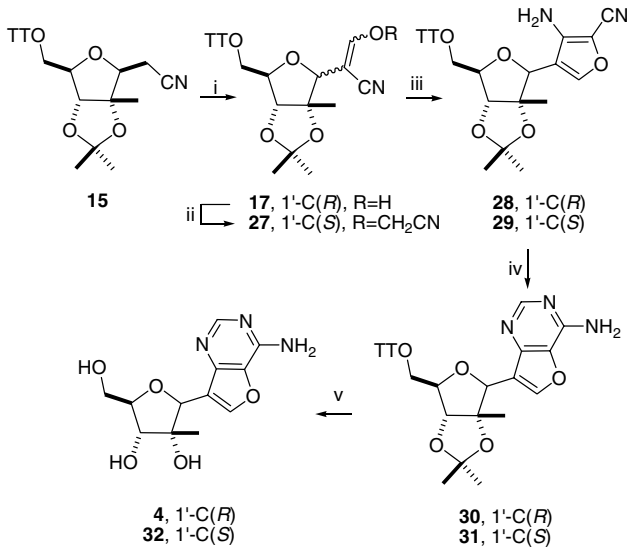
meric ratio of approximately 1–2 was in excellent agreement with that reported in the case of ribose.[27] The 1′-*C* chiral center established during the Moffatt procedure was expected to be isomerized at the subsequent step; therefore the choice of isomer **16** over **15** for further manipulations was arbitrary. The hydroxymethylene group in **17** can be introduced following the known[28] two-step procedure, but we found that this could be more readily accomplished by a direct Claisen condensation. Indeed, the carbanion derived from **16** reacted smoothly with methyl formate, and the pyrrole ring was easily constructed following a published[16] DBU-induced Kirsch type[29] cyclization. After the formation of the enamine **18**, the system was stabilized by *N*-acylation (**19**) which also greatly facilitated the DBU-induced cyclization. The isomeric pyrroles **20** and **21** (produced in a ratio of 1:5) were separated by column chromatography, and the stereochemistry at 1′-*C* was established by nOe experiments (isomers **22** and **24**).

The ethoxycarbonyl protecting group in **20** was hydrolyzed (**22**), and the pyrimidine ring was constructed following a published procedure.[16] The trityl and acetonide protecting groups in **23** were removed under acidic conditions to provide nucleoside **3**. A similar sequence was used to obtain the 1′-*C*-α isomer **26** (Scheme 3).

Intermediates **15** or **16** served as a convenient starting point[17] for the synthesis of the furan analog **4** (Scheme 4). The arbitrarily chosen β-isomer **15** was subjected to a Claisen condensation with methyl formate yielding **17** as a mixture of diastereoisomers. *O*-Alkylation of the enol **17** with chloroacetonitrile smoothly produced the enol ether **27**. In the absence of the stabilizing effect of the ethoxyurethane group, such as in **19**, the Kirsch cyclization was much less efficient, and the desired furans **28** and **29** were obtained in a combined yield of only 12%. The respective α- and β-isomers were separated, and the pyrimidine ring was constructed with formamidine. A one-pot acid-catalyzed cleavage of the protecting groups then completed the synthesis of nucleosides **4** and **32**.



Scheme 3. Reagents and conditions: (i) K_2CO_3, EtOH, rt, 1 h; (ii) formamidine acetate, EtOH, 90 °C, 5 h; (iii) HCl, dioxane, rt, 24 h.



Scheme 4. Reagents and conditions: (i) LDA, MeOCHO, THF, −78 °C, 2 h then rt 20 min; (ii) $ClCH_2CN$, Cs_2CO_3, DMF, rt, 18 h; (iii) LDA, −78 °C, 2 h; (iv) formamidine acetate, EtOH, 85 °C, 12 h; (v) HCl, dioxane, MeOH, rt, 2 h.

3. Methods

The ability of the nucleoside analogs to inhibit HCV RNA replication was assessed using the cell-based bicistronic replicon assay[30] as modified for RNA quantitation by RNase protection.[9] In the replicon assay the intracellular levels of replicon RNA are maintained by the activity of the virally encoded replication complex. Control assays titrating the inhibitory potency (EC_{50} value) of compounds **1** and **2** demonstrated results that were similar to previous work.[9,11]

The ability of adenosine deaminase (ADA) to convert compounds **1**–**4** to the corresponding 6-oxo analogs was determined in vitro. Two different reaction conditions were investigated.[12] The milder reaction condition I was developed that would only partially convert **1** to 2′-*C*-methyl-inosine. Reaction condition II contained a higher enzyme concentration, higher reaction temperature, and longer reaction times, to differentiate whether the nucleoside analog was a poor substrate or was stable to ADA. Under reaction condition II, **1** was completely converted to 2′-*C*-methyl-inosine.

Computer-aided conformational analyses were performed as described previously.[12] For analysis of electrostatic potentials, the compounds were optimized at the B3LYP/6-31G** level using Gaussian03.[31] Electrostatic potential was plotted on the electronic density surface at the B3LYP/6-31G** level using Spartan '04 version 1.0.3.[32]

4. Results and discussion

Our approach to finding NS5B polymerase nucleoside inhibitors with altered susceptibility to ADA-mediated metabolism was to vary the base in such way as to modulate the electrostatic potential at position 7 without

changing the overall shape and conformation of the molecule, thereby maximizing the probability of the nucleosides being recognized by the appropriate kinases and by NS5B polymerase. The conformational preferences of **1**–**4** were calculated using the MMFFs force field and a dielectric constant of 50. The variations to the base in **1**–**4** were found to have no profound effect on the molecules' calculated conformational preferences. In each case, the lowest-energy conformation was found to be that with the ribofuranose ring in a Northern pucker induced by the 2′-*C*-Me group with a pseudorotational angle *P* of 8.5–16.4 and pucker amplitude τ of 38.9–39.2, and with the glycosyl torsion angle in the *anti*-configuration. The calculated energy difference between Northern and Southern puckers differed little among the four compounds, with the Northern conformation preferred by 2.3–2.5 kcal/mol. The *syn*–*anti* energy difference varied somewhat, from 0.57 kcal/mol for compound **2** to 0.14 kcal/mol for **4**. Superposition of the lowest energy conformations shows very good alignment of the sugar rings with only slight variation in the base due to a change in bond angles in the base upon modification of 9-N to carbon (Fig. 2).

Given the excellent conformational overlap between highly active nucleosides **1** or **2** and the 9-deaza nucleoside **3**, we were surprised to find out that the pyrrole analog **3** displayed only rather weak anti-HCV activity (EC_{50} 59.7 μM, 72 h) when evaluated in the replicon assay. On the other hand, the furan analog **4** was moderately active (EC_{50} 22 μM) in a 24-h assay and showed about threefold improved potency (EC_{50} 8.1 μM) when incubated in the presence of replicon-containing cells for 72 h. The results are summarized in Table 1.

The apparent improvement of potency of the furan analog **4** in the 72-h replicon assay likely reflects an increase in the concentration of intracellularly generated 5′-triphosphate of **4**. This observation suggests that the most likely reason for low antiviral activity of **3** and **4** is the slow conversion of the parent nucleosides to their respective 5′-triphosphates.

The design of metabolically more robust nucleosides than adenosine or its 2′-*C*-methyl analog **1** was based on several assumptions: (a) the main degradation pathway of these compounds is 6-NH_2 deamination,[33] (b) the hydrogen-bond acceptor at base position 7 is critical for recognition by the protonated Asp296 of adenosine deaminase (ADA)[34], and (c) the capacity of the 7-heteroatom to accept a hydrogen bond is determined by the electron density at this position.

Table 1. Inhibitory activity of nucleoside analogs in the cell-based replicon assay [a]

Compound	EC_{50} (μM) replicon assay		CC_{50}[c] (μM)
	24 h[b]	72 h[b]	
1	0.25	0.2 (n = 3)	>100
2	0.3	0.11 (n = 3)	>100
3	>100	59.7 (n = 2)	>100
4	22 (n = 3)	8.1 (n = 3)	>100
Valopicitabine	7.45 (n = 1)	1.50 (n = 2)	>100

[a] Compound inhibitory potency was determined in Huh-7 cells harboring a neomycin^R-encoding replicon from HCV genotype 1b using an RNase protection assay, as described in Section 3.

[b] Length of time that the compound was incubated in the presence of replicon-containing cells, prior to the determination of viral RNA levels.

[c] Compound cytotoxicity as determined using an MTS assay, as described in Section 3.

To gauge the effect of variation in the base on electronic properties, ab initio calculations were performed on the 9-Me-substituted bases of **1**–**4** at the B3LYP/6-31** level. Inclusion of the ribose did not change the results substantially. Plots depicting the B3LYP/6-31G** electrostatic potential mapped onto the molecular electronic density are presented in Figure 3. The naturally occurring adenine base of **1** is much more electronegative at the 7-position relative to the furan **4**, suggesting that the furan will be a poorer hydrogen bond acceptor than adenine. These results are in good agreement with the findings of Nobeli et al.,[35] who calculated that furan is a much weaker H-bond acceptor than pyridine, due largely to its lower negative partial charge at the acceptor atom. Calculation of pyridine and furan at the same level of theory as used to calculate **1**–**4** gave similar partial charges as those found on 7-N of **1** and 7-O of **4**, respectively.

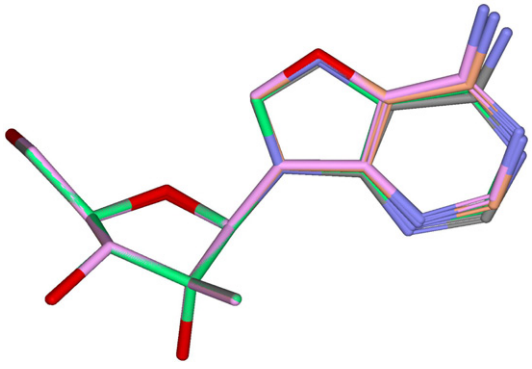


Figure 2. Overlaid lowest-energy minima (MMFFs, ε = 50) identified for **1**–**4**. Nitrogen atoms are shown in blue, oxygen atoms in red, and carbon atoms are color-coded by molecule: **1** (green), **2** (gray), **3** (orange), and **4** (magenta).

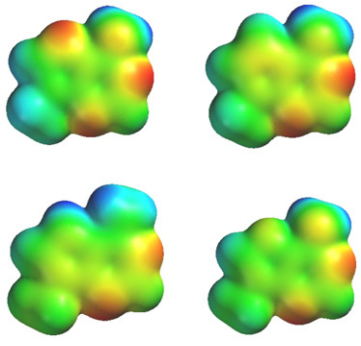


Figure 3. Electron density surfaces of the 9-Me-substituted bases from compounds **1**–**4** colored by the electrostatic potential (**1**, top left; **2**, top right; **3**, bottom left; **4**, bottom right; blue, electropositive; red, electronegative; green, neutral).

Table 2. Adenosine deaminase-mediated conversion of nucleoside analogs[a]

Compound	Reaction conditions I[b]		Reaction conditions II[c]	
	5 μM[d]	25 μM[d]	5 μM[d]	25 μM[d]
1	14	9	100	100
2	0	0	0	0
3	0	0	0	0
4	0	0	100	77

[a] Numbers represent the % of the analog converted to the corresponding 6-oxo analog under the assay conditions.
[b] Reactions included 0.00625 U/mL ADA and were carried out at 30 °C for 30 min. Under these conditions adenosine was 100% converted to inosine.
[c] Reactions included 1.25 U/mL ADA and were carried out at 37 °C for 24 h. Under these conditions adenosine was 100% converted to inosine.
[d] Concentration of the nucleoside analog in the reaction.

The observation that the hydrogen-bonding capacity at position 7 is weaker in **4** than in **1** and lacking altogether in **2** and **3** is consistent with the observed rates of deamination by ADA presented in Table 2. Compounds **2**–**4** were completely stable to conversion under reaction condition I, indicating that they are, at the least, less efficient substrates for ADA, whereas **2** and **3** were completely stable. Compound **4** was converted to the corresponding 6-oxo analog under reaction condition II, suggesting that nucleoside **4** is a better substrate for ADA compared to **2** and **3**.

In conclusion, novel 2′-*C*-methyl 9-deaza nucleosides **3** and **4** were successfully synthesized and their basic pharmacological properties evaluated. While the pyrrole derivative **3** was only weakly active, the 7-oxa analog **4** showed moderate anti-HCV activity in the standard 24- and 72-h replicon assay. The apparent improvement of the antiviral potency in the 72-h assay supports the hypothesis of a gradual intracellular buildup of the corresponding nucleoside 5′-*O*-triphosphates directly responsible for the inhibition of the NS5B polymerase. We have also demonstrated that the main degradation pathway of adenosines, 6-N deamination, can be effectively influenced by manipulation of electron density at position 7 of the heterobase.

5. Experimental

All non-hydrolytic reactions, unless indicated otherwise, were carried out in dry solvents purchased from Aldrich. HPLC analyses were performed at ambient temperature using a Waters XTerra C18, 5 μm, 50× 4.6 mm reversed phase column under following conditions: flow rate: 1.25 mL/min; eluent A: water (0.1% TFA), Eluent B: acetonitrile (0.1% TFA). Gradient: %B (time) starting at 10 (0) 50 (3), 90 (9), 100 (10), 100 (13), detector: UV at 220 nm. Analytical thin layer chromatography was performed on Silica Gel 60 F_{254} plates, 5× 10 cm, with a layer thickness of 250 μm and preparative TLC on Analtech Silica Gel GF plates, 20× 20 cm, with a layer thickness of 1000 μm. NMR spectra were recorded on a Varian Inova 500 or 600 MHz spectrometer. The chemical shifts (ppm) are positive in the low field direction and were referenced indirectly through the solvent's residual signal to TMS. Elemental analyses were performed at Robertson Microlit Laboratories, Madison NJ.

5.1. 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 after either 24- or 72-h incubation of the compound with the cells.[15] The replicon EC_{50} values are the average of at least two separate determinations (Table 1). The cytotoxicity of the nucleosides was determined by an MTS assay at 24 h.[15]

5.2. 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). Reactions were then initiated with addition of 25 μM nucleoside. Reaction mixtures 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). RP-HPLC analysis utilized a SUPELCOSIL LC-18-S column (Supelco 58931, 150× 4.6 mm, 5 μM) with 97.5%/2.5% 50 mM potassium phosphate, pH 4.4/MeOH (Buffer A) and 80%/20% 50 mM potassium phosphate, pH 4.4/MeOH (Buffer B), 1 mL/min flow rate. A 25 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, 10060% 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.

5.3. 1′-*O*-Methyl 2-*C*-methyl-3,5-bis-*O*-(4-methylbenzoyl)-α-D-ribofuranoside (6)

A solution of diol **5** (1.28 g, 3.19 mmol) and pyridinium *para*-toluenesulfonate (360 mg, 1.43 mmol) in anhydrous MeOH (6 mL) was heated to 80 °C in a sealed tube for 24 h. The solvent was evaporated and the residue was purified by flash chromatography (silica-gel, EtOAc/hexanes EtOAc: 0–60%) to yield 977 mg (74%) of the desired product as a single diastereoisomer. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.93 (d, J = 8.2 Hz, 2H), 7.90 (d, J = 8.2 Hz, 2H), 7.23 (d, J = 8.2 Hz, 2H), 7.14 (d, J = 8.2 Hz, 2H), 5.57 (d, J = 7.10 Hz, 1H), 4.73 (s, 1H), 4.58 (dd, J = 11.5, 3.9 Hz, 1H), 4.52 (m, 1H), 4.44 (dd, J = 11.7, 5.5 Hz, 1H), 3.37 (s, 3H), 2.41 (s, 3H), 2.36 (s, 3H), 1.37 (s, 3H). nOe (4 mg/mL, $CDCl_3$): irradiation of the singlet at 4.73 ppm (1′-*H*) resulted in a weak enhancement of the doublet at 5.57 ppm (3′-*H*) by <1%. ^{13}C NMR (500 MHz, $CDCl_3$) δ: 166.29, 165.58, 144.40, 143.54, 129.81, 129.68, 129.20, 128.91, 127.08, 126.28, 108.98, 79.49, 78.33, 77.11, 65.18, 55.18, 21.64, 21.57, 19.90. Mp: 107–109 °C. HRMS for $C_{23}H_{26}O_7$ calculated 437.1576 $[M+Na]^+$, found: 437.1584. CHN

analysis: for $C_{23}H_{26}NO_7$ calculated C, 66.65%; H, 6.32%; found: C, 66.68%; H, 6.54%.

5.4. 1′-*O*-Methyl-2′-*C*-methyl-2′,3′-*O*-(1-methylethylidene)-α-D-ribofuranoside (8)

A solution of the diester **6** (950 mg, 2.2922 mmol) in MeOH (10 mL) was treated with a methanolic solution of sodium methoxide (0.5 M, 3 mL), and stirring at room temperature was continued overnight. The reaction was quenched by addition of ethereal hydrogen chloride (1 N, 1.5 mL), and the volatiles were evaporated. The crude triol was dissolved in DCM (6 mL) and 2,2-dimethoxypropane (6 mL) was added, followed by a catalytic amount of *para*-toluenesulfonic acid. The reaction mixture was stirred at room temperature for 30 min and quenched by pouring onto a saturated solution of sodium bicarbonate (10 mL). The product was extracted with DCM (4× 30 mL), dried with anhydrous sodium sulfate, and evaporated to dryness. Purification by flash chromatography (silica-gel, EtOAc/hexanes EtOAc: 0–70%) afforded 345 mg (69%, two steps) of the desired product. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.81 (s, 1H), 4.44 (s, 1H), 4.26 (t, J = 3.0 Hz, 1H), 3.66 (dd, J = 12.4, 2.8 Hz, 1H), 3.58 (dd, J = 12.4, 3.2, 1H), 3.41 (s, 3H), 1.44 (s, 3H), 1.40 (s, 3H), 1.39 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$) δ: 112.40, 110.91, 92.00, 88.00, 87.30, 63.82, 55.86, 28.00, 27.57, 19.56. LCMS: for $C_{10}H_{18}O_5$ calculated 218.12, found: 241.30 $[M+Na]^+$. CHN analysis: for $C_{10}H_{18}O_5$ calculated C, 55.25%; H, 8.31%; found: C, 55.03%; H, 8.53%.

5.5. 1′-*O*-Methyl-2′-*C*-methyl-2′,3′-*O*-(1-methylethylidene)-5′-*O*-trityl-α-D-ribofuranoside (9)

A solution of alcohol **8** (612 mg, 2.80 mmol) in pyridine was treated with trityl chloride (782 mg, 2.80 mmol) and the reaction mixture was stirred at 60 °C overnight. It was poured into a 10% aqueous solution of citric acid (20 mL), and the crude product was extracted with *tert*-butyl methyl ether (3× 30 mL). The combined extracts were dried with anhydrous magnesium sulfate, filtered, and evaporated to dryness. The residue (1.3747 g) was purified by flash chromatography (silica-gel, EtOAc/hexanes, EtOAc: 0–30%) to yield 917 mg (71%) of the pure product. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.50 (m, 6H), 7.32 (m, 6H), 7.27 (m, 3H), 4.78 (s, 1H), 4.32 (dd, J = 8.0, 6.2 Hz, 1H), 4.23 (s, 1H), 3.27 (dd, J = 8.4, 6.0 Hz, 1H), 3.19 (s, 3H), 3.12 (t, J = 8.9 Hz, 1H), 1.51 (s, 3H), 1.44 (s, 3H), 1.25 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$) δ: 143.82, 128.60, 127.90, 127.78, 127.23, 127.02, 112.47, 110.62, 91.21, 88.23, 86.67, 84.64, 64.04, 55.11, 28.09, 27.71, 20.40. HRMS for $C_{29}H_{32}O_5$ calculated 483.2148 $[M+Na]^+$, found: 483.2132. CHN analysis: for $C_{29}H_{32}O_5$ calculated C, 75.63%; H, 7.00%; found: C, 75.58%; H, 7.15%.

5.6. 2′-*C*-Methyl-2′,3′-*O*-(1-methylethylidene)-5′-*O*-trityl-D-ribofuranose (10)

A solution of the allylether **14** (800 mg, 1.6441 mmol) and 1,3-bis(diphenylphosphino)propane nickel (II) dichloride (27 mg, 0.05 mmol) in diethyl ether (16 mL) was treated at 0 °C with Dibal-H (2 mL, 1 M solution in hexanes). Stirring at 0 °C was continued for 30 min and the reaction was quenched with 2 mL of water. It was stirred at ambient temperature for an additional 30 min, anhydrous magnesium sulfate was added (ca. 2 g), and the stirring was continued for another 30 min. The drying agent was filtered off, the solvent was evaporated, and the residue (795 mg) was purified by gradient chromatography (silica-gel, EtOAc/hexanes, EtOAc: 0–70%) to afford 536 mg (73%) of the desired product as mixture of epimers (ca. 1.2:1). ^{1}H NMR (500 MHz, CD_3Cl) major isomer (assignment based on integration and coupling pattern): δ: 7.30–7.40 (m, 12H), 7.25–7.30 (m, 3H), 5.22 (d, J = 8.0 Hz, 1H), 4.43 (br s, 1H), 4.27 (t, J = 4.10 Hz, 1H), 3.57 (d, J = 7.80 Hz, 1H), 3.41 (dd, J = 10.3, 3.9 Hz, 1H), 3.14 (dd, J = 10.1, 3.7 Hz, 1H), 1.49 (s, 3H), 1.46 (s, 6H). Minor isomer: δ: 7.30–7.40 (m, 12H), 7.25–7.30 (m, 3H), 5.14 (d, J = 10.5 Hz, 1H), 4.32 (br s, 1H), 4.24 (t, J = 3.7 Hz, 1H), 3.84 (d, J = 10.5 Hz, 1H), 3.35 (m, 2H), 1.56 (s, 3H), 1.46 (s, 6H). ^{13}C NMR (500 MHz, CD_3Cl) major isomer (assignment based on peak height): 142.97, 128.73, 127.97, 127.38, 112.62, 104.40, 92.04, 87.56, 87.19, 85.22, 64.76, 28.44, 27.83, 20.30. Minor isomer: 143.46128.68, 127.88, 127.13, 113.54, 101.92, 88.00, 87.76, 87.37, 80.91, 64.24, 27.31, 27.25, 22.04. HRMS for $C_{28}H_{30}O_5$ calculated 469.1991 $[M+Na]^+$, found: 469.1987. CHN analysis: for $C_{28}H_{30}O_5$ calculated C, 75.31%; H, 6.77%; found: C, 75.12%; H, 6.99%.

5.7. 1′-*O*-Allyl-2′-*C*-methyl-3′,5′-bis-*O*-(4-methylbenzoyl)-D-ribofuranoside (11)

A solution of 2′-*C*-methyl-3,5-bis-*O*-(4-methylbenzoyl)-D-ribofuranose (**5**) (2.00 g, 5.00 mmol) and pyridinium *para*-toluenesulfonate (627 mg, 2.5 mmol) in allyl alcohol (10 mL) was heated to 100 °C for 24 h. The volatile allyl alcohol was evaporated, and the residue was purified by gradient column chromatography (silica-gel, EtOAc/hexanes, EtOAc: 0–30%) to yield 3.55 g (81%) of the pure product. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.93 (d, J = 8.2 Hz, 2H), 7.88 (d, J = 8.0 Hz, 2H), 7.23 (d, J = 8.0 Hz, 2H), 7.15 (d, J = 8.0 Hz, 2H), 5.85 (m, 1H), 5.56 (d, J = 7.1 Hz, 1H), 5.26 (dd, J = 17.4, 1.8 Hz, 1H), 5.18 (dd, J = 10.5, 1.6 Hz, 1H), 4.88 (s, 1H), 4.43 to 4.60 (bm, 3H), 4.25 (ddt, J = 13.3, 5.0, 1.6 Hz, 1H), 4.0 (dt, J = 13.3, 5.7, 1.4 Hz, 1H), 2.42 (s, 3H), 2.28 (s, 3H), 1.41 (s, 3H). nOe (4 mg/mL, $CDCl_3$): irradiation of the singlet at 4.88 ppm (1′-*H*) resulted in a weak enhancement of the doublet at 5.56 ppm (3′-*H*) by <1%. ^{13}C NMR (500 MHz, $CDCl_3$) δ: 166.29, 165.57, 144.41, 143.54, 133.76, 129.83, 129.73, 129.22, 128.93, 127.02, 126.28, 119.98, 107.01, 79.64, 78.32, 77.16, 68.23, 65.22, 21.66, 21.59, 20.00. Mp: 64 °C. HRMS for $C_{25}H_{28}O_7$ calculated 463.1733 $[M+Na]^+$, found: 463.1727. CHN analysis: for $C_{25}H_{28}O_7$ calculated C, 68.17%; H, 6.41%; found: C, 68.15%; H, 6.43%.

5.8. 1′-*O*-Allyl-2′-*C*-methyl-D-ribofuranoside (12)

A solution of diester **11** (3.55 g, 8.059 mmol) was dissolved in anhydrous MeOH (20 mL) and treated with a methanolic solution of sodium methoxide (5 mL,

0.5 M solution). Stirring at room temperature was continued for 2 h, and the reaction was quenched by addition of 1N HCl (3 mL). The solvent was evaporated and the residue was purified by column chromatography (silica-gel, EtOAc, 100% EtOAc, isocratic) to obtain 1.1372 g (69%) of the desired product in the form of a white powder. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.88 (m, 1H), 5.29 (dd, J = 17.2, 1.4 Hz, 1H), 5.20 (dd, J = 10.3, 1.2 Hz, 1H), 4.80 (s, 1H), 4.23 (dd, J = 13.0, 5.0 Hz, 1H), 4.02 (m, 3H), 3.82 (dd, J = 11.7, 2.5 Hz, 1H), 3.66 (dd, J = 11.9, 3.7 Hz, 1H), 1.33 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$) δ: 133.73, 117.46, 107.24, 83.64, 79.18, 74.86, 68.97, 62.97, 19.33. Mp: 66–71 °C. HRMS for $C_9H_{16}O_5$ calculated 227.0895 $[M+Na]^+$, found: 227.0872. CHN analysis: for $C_9H_{16}O_5$ calculated C, 52.93%; H, 7.90%; found: C, 52.84%; H, 7.81%.

5.9. 1′-*O*-Allyl-2′-*C*-methyl-2′,3′-*O*-(1-methylethylidene)-D-ribofuranoside (13)

A solution of the triol **12** (1.125 g, 5.51 mmol) in DCM (6 mL) was treated with 2,2-dimethoxypropane (4 mL) and *para*-toluenesulfonic acid (187 mg), and stirred at room temperature for 2 h. The reaction mixture was then poured onto aqueous solution of sodium bicarbonate (20 mL) and extracted with DCM (3× 50 mL). The combined extracts were dried, and the solvent was distilled off at 100 mm Hg pressure at ambient temperature. The resulting volatile product was used in the next step as obtained. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.90 (m, 1H), 5.30 (dd, J = 15.6, 1.4 Hz, 1H), 5.22 (dd, J = 10.5, 1.4 Hz, 1H), 4.49 (s, 1H), 4.26 (m, 2H), 3.69 (dd, J = 12.4, 2.5 Hz, 1H), 3.61 (dd, J = 12.4, 3.7 Hz, 1H), 1.46 (br s, 6H), 1.41 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$) δ: 133.20, 117.92, 112.45, 108.93, 92.23, 88.18, 87.39, 69.23, 63.89, 28.05, 27.63, 19.76.

5.10. 1′-*O*-Allyl-2′-*C*-methyl-2′,3′-*O*-(1-methylethylidene)-5′-*O*-trityl-D-ribofuranoside (14)

A solution of alcohol **13** (1.77 g, max. 5.51 mmol) and trityl chloride (1.536 g, 5.51 mmol) in pyridine (6 mL) was stirred at 60 °C overnight. The solvent was evaporated and the residue was purified by gradient chromatography (silica-gel, EtOAc/hexanes EtOAc: 0–30%) to yield 1.4554 g (54%, two steps) of the desired product. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.48 (m, 6H), 7.30 (m, 9H), 5.65 (m, 1H), 5.08(m, 2H), 4.34 (dd, J = 8.5, 5.5 Hz, 1H), 4.00 (dd, J = 13.3, 4.3 Hz, 1H), 3.84 (dd, J = 13.3, 5.5 Hz, 1H), 3.28 (dd, J = 9.4, 5.5 Hz, 1H), 3.10 (t, J = 9.4 Hz, 1H), 1.51 (s, 3H), 1.45 (s, 3H), 1.28 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$) δ: 143.81, 128.64, 127.77, 126.99, 116.25, 112.45, 108.53, 91.42, 88.37, 86.68, 84.66, 67.94, 64.06, 28.10, 27.71. HRMS for $C_{31}H_{34}O_5$ calculated 509.2304 $[M+Na]^+$, found: 509.2290. CHN analysis: for $C_{31}H_{34}O_5$ calculated C, 76.52%; H, 7.04%; found: C, 76.49%; H, 7.29%.

5.11. 2′-*C*-Methyl-2′,3′-*O*-(1-methylethylidene)-5′-*O*-trityl-α-D-ribofuranos-1′(*S* or *R*)-yl-acetonitrile (15, 16)

A stirred suspension of sodium hydride (120 mg, 3.0 mmol, 60% in mineral oil) in dimethoxyethane (DME, 5 mL) was cooled to 0 °C. A solution of diethyl cyanomethylphosphonate (550 μL, 3.40 mmol) in DME was added dropwise, and the stirring was continued for 10 min at 0 °C. A solution of the sugar **10** (760 mg, 1.70 mmol) in DME (5.0 mL) was then added, cooling was removed, and stirring continued for 4 h. The reaction was quenched with water and extracted with *tert*-butyl methyl ether (3×). The combined organic extracts were washed with brine, dried with anhydrous magnesium sulfate, and filtered, and the solvent was evaporated. The crude product (909 mg) was purified by gradient chromatography (silica-gel, EtOAc/hexanes, EtOAc: 0–50%). Two epimers were obtained: minor epimer (**15**): 180 mg (23%) ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.48 (m, 6H), 7.34 (m, 6H), 7.28 (m, 3H), 4.20 (m, 2H), 4.05 (dd, J = 7.8, 5.3 Hz, 1H), 3.33 (d, J = 4.1 Hz, 1H), 2.61 (dd, J = 16.7, 5.3 Hz, 1H), 2.52 (dd, J = 16.7, 8.0 Hz, 1H), 1.55 (s, 3H), 1.38 (s, 3H), 1.37 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$) δ: 143.58, 128.73, 127.83, 127.12, 116.95, 114.56, 87.61, 87.32, 87.00, 82.28, 80.94, 63.52, 28.11, 26.62, 18.69, 18.39. HRMS for $C_{30}H_{31}NO_4$ calculated 492.2151 $[M+Na]^+$, found: 492.2149. CHN analysis: for $C_{30}H_{31}NO_4$ calculated C, 76.73%; H, 6.65%; N, 2.98%; found: C, 76.68%; H, 6.80%; N, 2.81%. Major epimer (**16**): 390 mg (49%) ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.46 (m, 6H), 7.35 (m, 6H), 7.27 (m, 3H), 4.43 (d, J = 0.9 Hz, 1H), 4.25 (t, J = 4.80 Hz, 1H), 4.03 (dd, J = 7.3, 5.7 Hz, 1H), 3.29 (dd, J = 10.1, 5.3 Hz, 1H), 3.22 (dd, J 10.3, 4.6 Hz, 1H), 2.68 (ABq, J = 16.7, 7.3 Hz, 2H), 1.50 (s, 3H), 1.44 (s, 3H), 1.43 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$) δ: 143.37, 128.62, 127.94, 127.21, 117.59, 113.32, 89.21, 89.07, 87.39, 83.56, 81.62, 63.68, 27.73, 27.32, 22.98, 27.32, 22.97, 18.60. HRMS for $C_{30}H_{31}NO_4$ calculated 492.2151 $[M+Na]^+$, found: 492.2139. CHN analysis: for $C_{30}H_{31}NO_4$ calculated C, 76.73%; H, 6.65%; N, 2.98%; found: C, 76.69%; H, 6.83%; N, 2.97%.

5.12. (1′*S* or 1′*R*)-1-[4-amino-5-cyano-1-(ethoxycarbonyl)-1*H*-pyrrol-3-yl]-1,4-anhydro-2′-*C*-methyl-2,3-*O*-(1-methylethylidene)-5′-*O*-trityl-D-ribitol (20, 21)

A solution of diisopropyl amine (1.42 mL, 10 mmol) in THF (40 mL) was cooled to −78 °C and treated dropwise with *n*-butyllithium (4.00 mL, 10 mmol, 2.5 M solution in hexanes). To this mixture, a solution of **16** (2.22 g, 4.78 mmol) in THF (60 mL) was added at −78 °C, via syringe. Stirring at cold was continued for 90 min, after which time neat methyl formate (1.00 mL, 16.20 mmol) was added. The reaction mixture was stirred at −78 °C for another 2 h, and the dry ice cooling bath was replaced with an ice bath. After 20 min the reaction was quenched by pouring onto a 10% aqueous solution of citric acid. The product was extracted with $CHCl_3$ (4× 100 mL), the combined extracts were washed with brine (1× 100 mL) and dried (anhydrous sodium sulfate), and the solvent was evaporated. The crude **17** (2.67 g) was dissolved in MeOH (20 mL), and water was added (2.0 mL) followed by sodium acetate (540 mg, 6.60 mmol) and aminoacetonitrile hydrochloride (610 mg, 6.60 mmol). The reaction mixture was stirred at ambient temperature for 72 h. It was diluted with $CHCl_3$ and washed with water. The aqueous

phase was back-washed with $CHCl_3$, the combined organic phase was dried, and the solvent was evaporated. The crude product (**18**, 2.44 g) was dissolved in DCM (20 mL) and DBU (1.34 g, 8.80 mmol) was added. To this mixture ethyl chloroformate (716 μL, 6.60 mmol) was added via syringe, and stirring at room temperature was continued for 2 h. At this time, LC–MS indicated full formation of the urethane **19**, and additional DBU (1.34 g, 8.80 mmol) was added to induce the cyclization. The stirring at room temperature was continued overnight. The reaction mixture was diluted with $CHCl_3$ (50 mL) and extracted with an aqueous solution of citric acid (10%, 2× 50 mL). The combined aqueous phases were washed with $CHCl_3$, the combined organic extracts were dried (anhydrous sodium sulfate), and the solvent was evaporated. The crude product (2.38 g) was purified by column chromatography (silica-gel, dichloromethane/diethyl ether 98:2, isocratic) to obtain two epimers: minor epimer (**20**, higher Rf, 280 mg, 12%): ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.52, (m, 6H), 7.36 (m, 6H), 7.30 (m, 3H), 4.76 (s, 1H), 4.57 (br s, 2H), 4.44 (q, J = 7.1 Hz, 2H), 4.40 (d, J = 2.50 Hz, 1H), 4.32 (dd, J = 6.7, 3.9 Hz, 1H), 3.43 (dd, J = 10.5, 3.5 Hz, 1H), 3.36 (dd, J = 10.5, 4.5 Hz, 1H), 1.63 (s, 3H), 1.47 (t, J = 7.1 Hz, 2H), 1.43 (s, 3H), 1.24 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$) δ: 146.11, 143.57, 128.72, 127.81, 127.15, 122.20, 114.69, 89.15, 86.95, 86.90, 82.68, 81.68, 64.18, 63.78, 31.54, 28.08, 26.50, 22.60, 19.62, 14.10, 14.07. HRMS for $C_{36}H_{37}N_3O_6$ calculated 630.2580 $[M+Na]^+$, found: 630.2595. CHN analysis: for $C_{36}H_{37}N_3O_6$ calculated C, 71.15%; H, 6.14%; N, 6.91%; found: C, 71.48%; H, 6.45%; N, 6.59%. Major epimer (**21**, lower Rf, 1.25 g, 56%): ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.48 (m, 6H), 7.34 (m, 6H), 7.26 (m, 3H), 5.31 (s, 1H), 4.71 (s, 1H), 4.47 (m, 3H), 4.33 (t, J = 5.0 Hz, 1H), 3.33 (dd, J = 10.3, 5.3 Hz, 1H), 3.27 (dd, J = 10.3, 5.0 Hz, 1H), 1.57 (s, 3H), 1.45 (m, 9H). ^{13}C NMR (500 MHz, $CDCl_3$) δ: 143.39, 128.61, 127.94, 127.25, 123.55, 123.00, 90.40, 88.07, 87.35, 83.19, 82.59, 64.29, 63.37, 27.63, 27.53, 14.08. HRMS for $C_{36}H_{37}N_3O_6$ calculated 630.2580 $[M+Na]^+$, found: 630.2570. CHN analysis: for $C_{36}H_{37}N_3O_6$ calculated C, 71.15%; H, 6.14%; N, 6.91%; found: C, 71.20%; H, 6.11%; N, 6.65%.

5.13. (1′*S*)-1-(4-Amino-5-cyano-1*H*-pyrrol-3-yl)-1,4-anhydro-2′-*C*-methyl-2′,3′-*O*-(1-methylethylidene)-5-*O*-trityl-D-ribitol (22)

A solution of the urethane **20** (207 mg, 0.341 mmol) in EtOH (6 mL) was treated with potassium carbonate (100 mg) and stirred at ambient temperature for 1 h. The reaction was quenched with water and extracted with $CHCl_3$/iPrOH (85:15, 3×). The combined organic extracts were dried with anhydrous sodium sulfate and the solvent was evaporated. The crude product (182 mg, 100%) was used in the next step without additional purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.0 (s, 1H), 7.56 (m, 6H), 7.33 (m, 6H), 7.27 (m, 3H), 6.68 (d, J = 2.1 Hz, 1 H), 4.82 (s, 1H), 4.33 (d, J = 2.80 1H), 4.29 (m, 1H), 4.12 (s, 2H), 3.42 (dd, J = 10.3, 3.9 Hz, 1H), 3.35 (dd, J = 10.3, 4.6 Hz, 1H), 1.63 (s, 3H), 1.41 (s, 3H), 1.21 (s, 3H). nOe (600 MHz, 1 mg/mL, $CDCl_3$): irradiation of the singlet at 5.22 ppm (1′-*H*) resulted in a pronounced enhancement of the multiplet at 4.29 ppm (4′-*H*) by 4%. ^{13}C NMR (500 MHz, $CDCl_3$) δ: 143.68, 141.72, 128.73, 127.83, 127.13, 120.84, 114.94, 114.51, 109.47, 89.54, 87.04, 86.89, 86.43, 82.59, 82.41, 63.71, 31.57, 28.21, 26.69, 22.63, 19.80, 14.10. HRMS for $C_{33}H_{33}N_3O_4$ calculated 558.2369 $[M+Na]^+$, found: 558.2374. CHN analysis: for $C_{33}H_{33}N_3O_4$ calculated C, 74.00%; H, 6.21%; N, 7.84%; found: C, 73.75%; H, 6.93%; N, 7.06%.

5.14. (1′*S*)-1-(4-Amino-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-1,4-anhydro-2-*C*-methyl-2′,3′-*O*-(1-methyl-ethylidene)-5′-*O*-trityl-D-ribitol (23)

A solution of the nitrile **22** (182 mg, 0.339 mmol) and formamidine acetate (354 mg, 3.40 mmol) in EtOH (6 mL) was heated with stirring in a sealed tube to 90 °C for 5 h. After cooling to ambient temperature, the solvent was evaporated. Water was added, and the product was extracted with *tert*-butyl methyl ether (4×). The combined organic extracts were back washed with brine and dried, and the solvent was evaporated to yield 173 mg of crude product. It was further purified by preparative TLC (DCM + MeOH/9:1) to obtain 137 mg of pure product. ^{1}H NMR (500 MHz, CD_3OD) δ: 8.16 (s, 1H), 7.50 (m, 6H), 7.32 (m, 6H), 7.26 (m, 4H), 5.29 (s, 1H), 4.30 (d, J = 3.0 Hz, 1H), 4.23 (m, 1H), 3.40 (dd, J = 5.0, 1.6 Hz, 2H), 1.64 (s, 3H), 1.38 (s, 3H), 1.15 (s, 3H). ^{13}C NMR (500 MHz, CD_3OD) δ: 149.92, 145.22, 129.95, 129.90, 129.19, 128.88, 128.25, 115.84, 112.41, 90.66, 89.07, 88.26, 83.31, 83.22, 65.27, 28.59, 27.29, 21.77. HRMS for $C_{34}H_{34}N_4O_4$ calculated 563.2658 $[M+H]^+$, found: 563.2662. CHN analysis: for $C_{34}H_{34}N_4O_5$ (0.5 H_2O) calculated C, 71.43%; H, 6.17%; N, 9.80%; found: C, 71.51%; H, 6.00%; N, 8.52%.

5.15. (1′*S*)-1-(4-Amino-5*H*-pyrrolo[3,2-d]pyrimidin-7-yl)-1,4-anhydro-2′-*C*-methyl-D-ribitol (3)

A solution of the protected nucleoside **23** (105 mg, 0.1966 mmol) was dissolved in MeOH (2.0 mL, anhydrous) and treated with a solution of hydrogen chloride in dioxane (4 N, 200 μL). Stirring at ambient temperature was continued for 24 h, after which the solvent was evaporated. Water was added, and the product was extracted with *tert*-butyl methyl ether (3×). The aqueous phase was heated briefly to reflux with charcoal, micro-filtered, and the solvent was evaporated to dryness. The residue was triturated with 2 mL of acetonitrile. The solid was washed two more times with cold acetonitrile and dried on high-vacuum to afford 33.7 mg (61%) of the desired product. ^{1}H NMR (600 MHz, CD_3OD) δ: 8.36 (s, 1H, 2-*H*), 7.73 (s, 1H, 8-*H*), 5.11 (s, 1H, 1′-*H*), 4.05 (dd, J = 11.8, 2.5 Hz, 1H, 5″-*H*), 3.97 (dt, J = 7.7, 2.5 Hz, 1H, 4′-*H*), 3.93 (dd, J = 11.7, 2.6 Hz, 5′-*H*), 3.82 (d, J = 7.6 Hz, 1H, 3′-*H*), 0.92 (s, 3H, C_2'-CH_3). ^{13}C NMR (600 MHz, CD_3OD) δ: 154.50 (C_6), 145.91 (C_8), 133.59, 130.49 (C_2), 84.66 (C_1'), 83.87 (C_4'), 79.74 (C_2'), 75.36 (C_3'), 61.16 (C_5'), 22.60 (C_2'-CH_3). HRMS for $C_{12}H_{16}N_4O_5$ calculated 281.1250 $[M+Na]^+$, found: 281.1265.

5.16. (1′*R*)-1-(4-amino-5-cyano-1*H*-pyrrol-3-yl)-1,4-anhydro-2′-*C*-methyl-2′,3′-*O*-(1-methylethylidene)-5-*O*-trityl-D-ribitol (24)

A solution of the urethane **11** (1.076 g, 1.77 mmol) in EtOH (20 mL) was treated with potassium carbonate (500 mg) and stirred at ambient temperature for 1 h. Water was added and the mixture was extracted with $CHCl_3$/iPrOH (85:15, 3× 50 mL). The combined organic extracts were dried with anhydrous sodium sulfate, and the solvent was evaporated. The crude product (995 mg, ~100%) was purified by gradient chromatography (silica-gel, EtOAc/hexanes, EtOAc: 0–100%) to obtain 704 mg (74%) of pure product. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.36 (s, 1H), 7.48 (d, J = 7.3 Hz, 6H), 7.34 (t, J = 7.3 Hz, 6H), 7.28 (t, J = 7.1 Hz, 3H), 6.76 (d, J = 3.44 Hz, 1H), 4.77 (s, 1H), 4.51 (d, J = 1.1 Hz, 1H), 4.29 (t, J = 4.8 Hz, 1H), 3.36 (dd, J = 10.1, 5.3 Hz, 1H), 3.30 (dd, J = 10.1, 5.0 Hz, 1H), 2.00 (s, 2H), 1.54 (s, 3H), 1.46 (s, 3H), 1.44 (s, 3H). nOe (2 mg/mL, $CDCl_3$): irradiation of the singlet at 4.78 ppm (1′-*H*) resulted in a weak enhancement of the doublet at 4.48 ppm (3′-*H*) by <1%. ^{13}C NMR (600 MHz, CD_3OD) δ: 143.49, 128.59, 127.88, 127.15, 123.09, 114.92, 112.86, 107.61, 90.49, 88.29, 87.19, 87.10, 82.79, 81.88, 63.71, 28.11, 27.79, 22.27. HRMS for $C_{33}H_{33}N_3O_4$ calculated 558.2369 $[M+Na]^+$, found: 558.2350. CHN analysis: for $C_{33}H_{33}N_3O_4$ calculated C, 74.00%; H, 6.21%; N, 7.84%; found: C, 73.73%; H, 5.93%; N, 7.70%.

5.17. (1*R*)-1-(4-Amino-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-1,4-anhydro-2-*C*-methyl-2,3-*O*-(1-methyl ethylidene)-5-*O*-trityl-D-ribitol (25)

A solution of the nitrile **24** (700 mg, 1.307 mmol) and formamidine acetate (1.36 g, 13.07 mmol) in EtOH (10 mL) was heated with stirring in a sealed tube to 90 °C for 3 h. The solvent was evaporated and water (30 mL) was added. The product was extracted with *tert*-butyl methyl ether (4× 250 mL), the combined extract was washed with brine (1× 50 mL), dried (anhydrous sodium sulfate), and the solvent was evaporated. The crude product (705 mg) was purified by gradient chromatography (silica-gel, MeOH/DCM, MeOH: 0–15%) to obtain 607 mg (82%) of pure product. ^{1}H NMR (500 MHz, CD_3CN) δ: 9.62 (s, 1H), 8.21 (s, 1H), 7.50 (m, 6H), 7.33 (m, 6H), 7.26 (m, 3H), 5.68 (s, 1H), 5.28 (s, 1), 4.36 (d, J = 0.7 Hz, 1H), 4.18 (t, J = 5.72 Hz, 1H), 3.34 m (dd, J = 10.1, 6.4 Hz, 1H), 3.20 (dd, J = 10.7, 5.5, 1H), 1.49 (s, 3H), 1.34 (s, 3H), 1.33 (s, 3H). ^{13}C NMR (500 MHz, CD_3CN) δ: 151.61, 151.08, 147.75, 144.93, 129.75, 129.60, 128.93, 128.15, 118.29, 114.17, 113.18, 112.11, 90.84, 89.76, 87.97, 83.66, 80.35, 63.98, 28.44, 28.08, 23.08. HRMS for $C_{34}H_{34}N_4O_4$ calculated 563.2658 $[M+H]^+$, found: 563.2662. CHN analysis: for $C_{34}H_{34}N_4O_4$ calculated C, 72.58%; H, 6.09%; N, 9.96%; found: C, 72.70%; H, 6.16%; N, 9.72%.

5.18. (1′*R*)-1-(4-Amino-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-1,4-anhydro-2′-*C*-methyl-D-ribitol (26)

A solution of **25** (148 mg, 0.263 mmol) in MeOH (2.0 mL, anhydrous) was treated with a solution of hydrogen chloride in dioxane (4 N, 200 μL) and stirred at ambient temperature for 24 h. The solvent was evaporated and the residue was dissolved in water. After extraction with DCM (3×) the combined organic extracts were washed with water (1×). The combined aqueous phase was decolorized with charcoal and evaporated to dryness. The crude product (98 mg) was recrystallized from acetonitrile to yield 56 mg (77%) of the pure product. ^{1}H NMR (500 MHz, CD_3OD) δ: 8.36 (s, 1H, 2-*H*), 7.75 (s, 1H, 8-*H*), 4.95 (s, 1H, 1′-*H*), 4.17 m (1H, 4′-*H*), 4.06 (d, J = 8.0 Hz, 1H, 3′-*H*), 3.86 (dd, J = 11.9, 1.6 Hz, 1H, 5″-*H*), 3.70 (dd, J = 11.9, 4.4 Hz, 1H, 5′-*H*), 1.19 (s, 3H, C'_2-CH_3). ^{13}C NMR (600 MHz, CD_3OD) δ: 154.50 (C_6), 145.90 (C_7), 135.32, 132.11 (C_2), 114.88, 109.97, 83.39 (C'_4), 82.13 (C'_1), 79.66 (C'_2), 77.46 (C'_3), 63.34 (C'_5), 20.31 (C'_2-CH_3). HRMS for $C_{12}H_{16}N_4O_5$ calculated 281.1250 $[M+Na]^+$, found: 281.1249.

5.19. (1′*S* or 1′*R*)-1-(4-Amino-5-cyano-3-furyl)-1,4-anhydro-2′-*C*-methyl-2′,3′-*O*-(1- methylethylidene)-5-*O*-trityl-D-ribitol (28, 29)

The solution of the hydroxymethylene derivative **17** (260 mg, max. 0.50 mmol) in DMF (4 mL) was treated with chloroacetonitrile (300 μL, large excess), cesium carbonate (700 mg) was added and stirred at ambient temperature overnight. The reaction mixture was poured into water (20 mL) and extracted with *tert*-butyl methyl ether (3× 30 mL). The combined organic phase was washed with brine and dried (anhydrous sodium sulfate) and the solvent was evaporated to dryness. The crude **27** (222 mg) was dissolved in THF (4 mL) and added drop-wise into a solution of LDA [prepared from *n*Buli (2.5 M in hexanes, 2.1 mL, 5.20 mmol) and diisopropylamine (730 μL, 5.20 mmol)] at −78 °C via syringe. The reaction mixture was stirred at −78 °C for 2 h and was quenched with a saturated solution of ammonium chloride (20 mL). The crude product was extracted with *tert*-butyl methyl ether (4× 30 mL), the combined organic extract was washed with brine (1× 30 mL) and dried with anhydrous magnesium sulfate, and the solvent was evaporated. The crude product was purified by gradient chromatography (silica-gel, EtOAc/hexanes, EtOAc: 0–70%) to obtain 17.2 mg (3%) of the β-epimer **28** and 73 mg (9%) of α-epimer **29**. Minor epimer (**28**): ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.48 (m, 6H), 7.34 (m, 6H), 7.28 (m, 3H), 7.22 (s, 1H), 4.74 (s, 1H), 4.31 (m,3H), 3.41 (dd, J = 10.3, 3.9 Hz, 1H), 3.36 (dd, J = 10.5, 4.6 Hz, 1H), 1.62 (s, 3H), 1.41 (s, 3H), 1.24 (s, 3H). ^{13}C NMR (600 MHz, $CDCl_3$) δ: 143.61, 143054, 143.44, 128.70, 127.85, 127.20, 115.43, 114.76, 112.81, 89.00, 87.00, 86.91, 82.77, 81.00, 63.54, 29.67, 28.11, 26.53, 19.55. HRMS for $C_{33}H_{32}N_2O_5$ calculated 559.2203 $[M+Na]^+$, found: 559.2240. Major epimer (**29**): ^{1}H NMR (500 MHz, CD_3OD) δ: 7.48 (m, 6H), 7.35 (m, 6H), 7.30 (m, 4H), 4.74 (s, 1H), 4.52 (d, J = 0.7 Hz, 1H), 4.35 (m, 3H), 3.38 (dd, J = 10.3, 5.0 Hz, 1H), 3.30 (dd, J = 10.1, 5.0 Hz, 1H), 1.52 (s, 3H), 1.47 (s, 3H), 1.46 (s, 3H). ^{13}C NMR (600 MHz, CD_3OD) δ: 145.62, 144.69, 143.36, 128.67, 128.58, 127.92, 127.82, 127.24, 127.17, 113.84, 113.13, 112.76, 110.41, 90.34, 88.12, 87.33, 83.35, 80.87, 63.48, 31.52,

27.83, 27.56, 22.40. HRMS for $C_{33}H_{32}N_2O_5$ calculated 559.2203 $[M+Na]^+$, found: 559.2186.

5.20. (1′*S*)-1-(4-aminofuro[3,2-*d*]pyrimidin-7-yl)-1,4-anhydro-2′-*C*-methyl-2′,3′-*O*-(1-methylethylidene)-5-*O*-trityl-D-ribitol (30)

A solution of the amino nitrile **28** (37 mg, 0.069 mmol) in EtOH (3 mL) was treated with formamidine acetate (214 mg, 2.07 mmol) and heated to 85 °C in a sealed tube for 12 h. The solvent was evaporated, and the crude product was purified by preparative TLC (DCM + MeOH/9:1) to obtain 14.0 mg (36%) of the desired product. ^{1}H NMR (500 MHz, CD_3OD) δ: 8.24 (s, 1H), 7.88 (s, 1H), 7.50 (m, 6H), 7.31 (m, 6H), 7.25 (m, 3H), 5.17 (s, 1H), 4.30 (d, J = 3.0 Hz, 1H), 4.25 (m, 1H), 3.34 (d, J = 5.04 Hz, 2H), 1.63 (s, 3H), 1.37 (s, 3H), 1.17 (s, 3H). ^{13}C NMR (500 MHz, CD_3OD) δ: 154.42, 148.51, 145.23, 129.95, 128.87, 128.25, 120.04, 115.85, 90.70, 89.18, 88.23, 83.78, 82.65, 65.21, 28.64, 27.31, 21.69. HRMS for $C_{34}H_{33}N_3O_5$ calculated 586.2312 $[M+Na]^+$, found: 586.2302.

5.21. (1′*S*)-1-(4-Aminofuro[3,2-*d*]pyrimidin-7-yl)-1,4-anhydro-2-*C*-methyl-D-ribitol (4)

A solution of the protected nucleoside **30** (15.7 mg, 0.028 mmol) in MeOH (3 mL) was treated with a solution of HCl in dioxane (4 N, 1 mL) and stirred at ambient temperature for 2 h. The solvent was evaporated, and the residue was partitioned between water (10 mL) and DCM (20 mL). The aqueous phase was extracted with DCM (2×), treated with charcoal, and filtered through Celite. The filtrate was concentrated to a volume of about 5 mL, micro-filtered, and evaporated to dryness. Trituration with acetonitrile afforded 7.0 mg (88%) of the clean nucleoside. ^{1}H NMR (500 MHz, CD_3OD) δ: 8.47 (s, 1H), 8.26 (s, 1H), 5.09 (s, 1H), 4.02 (d, J = 13.0 Hz, 1H), 3.96 (br s, 1H), 3.87 (d, J = 11.7 Hz, 1H), 3.82 (d, J = 7.4 Hz, 1H), 1.00 (s, 3H). ^{13}C NMR (600 MHz, CD_3OD) δ: 153.27, 150.19, 149.18, 118.68, 84.55, 82.36, 79.56, 75.40, 61.43, 22.33. HRMS for $C_{12}H_{15}N_3O_5$ calculated 281.1012, found: 282.1044. $[M+H]^+$.

5.22. (1′*R*)-1-(4-Aminofuro[3,2-*d*]pyrimidin-7-yl)-1,4-anhydro-2′-*C*-methyl-2′,3′-*O*-(1-methyl ethylidene)-5-*O*-trityl-D-ribitol (31)

A solution of the amino nitrile **29** (93 mg, 00.173 mmol) in EtOH (3 mL) was treated with formamidine acetate (243 mg, 2.33 mmol) and heated to 85 °C in a sealed tube for 12 h. The solvent was removed in vacuo, and the crude product (54.1 mg) was purified by preparative TLC (DCM + MeOH/9:1) to obtain 23.9 mg (25%) of the desired product. ^{1}H NMR (500 MHz, CD_3OD) δ: 8.25 (s, 1H), 8.00 (s, 1H), 7.43 (m, 6H), 7.31 (m, 6H), 7.23 (m, 3H), 5.12 (s, 1H), 4.43 (s, 1H), 4.25 (t, J = 6.0 Hz, 1H), 3.36 (dd, J = 10.1, 6.0 Hz, 1H), 3.24 (m, 2H), 1.45 (s, 3H), 1.44 (s, 3H), 1.35 (s, 3H). ^{13}C NMR (500 MHz, CD_3OD) δ: 154.28, 150.25, 145.08, 129.89, 128.96, 128.28, 118.54, 110.18, 91.23, 90.40, 88.56, 84.73, 80.60, 63.97, 28.28, 27.93, 23.27. HRMS for $C_{33}H_{32}N_2O_5$ calculated 559.2203 $[M+Na]^+$, found: 559.2246.

5.23. (1′*R*)-1-(4-Aminofuro[3,2-*d*]pyrimidin-7-yl)-1,4-anhydro-2′-*C*-methyl-D-ribitol (32)

A solution of the protected nucleoside **31** (23 mg, 0.041 mmol) was dissolved in MeOH (3.0 mL), treated with 1.0 mL of 4 N solution of HCl in dioxane, and stirred at ambient temperature for 24 h. The reaction mixture was distributed between water (10 mL) and DCM (20 mL), and the aqueous phase was extracted with DCM (2×). The combined organic extract was washed with water (6 mL) and the combined aqueous phase was decolorized with charcoal and filtered through Celite. The filtrate was concentrated to a volume of about 5 mL, micro-filtered, and the remaining solvent was evaporated to dryness. The residue was recrystallized from acetonitrile to afford 8.4 mg (73%) of the desired nucleoside. ^{1}H NMR (500 MHz, CD_3OD) δ: 8.49 (s, 1H), 8.32 (s, 1H), 4.96 (s, 1H), 4.19 (br s, 1H), 4.04 (d, J = 7.8 Hz, 1H), 3.84 (d, J = 9.6 Hz, 1H), 3.68 (dd, J = 11.9, 3.4 Hz, 1H), 1.22 (s, 3H). ^{13}C NMR (500 MHz, CD_3OD) δ: 151.43, 148.29, 83.79, 80.14, 77.27, 63.17, 40.05, 20.27. ^{13}C NMR (500 MHz, CD_3OD) δ: 153.27, 150.19, 149.18, 118.68, 84.55, 82.37, 79.56, 75.40, 22.34. HRMS for $C_{12}H_{15}N_3O_5$ calculated 281.1012, found: 282.1018. $[M+H]^+$.

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